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Retrospective analysis of the effects of *Blastomyces* antigen concentration in urine and radiographic findings on survival in dogs with blastomycosis

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Abstract

Background: The *Blastomyces* antigen concentration in urine (BACU) test is used to diagnose blastomycosis and monitor treatment in dogs. It is unknown if a higher BACU is associated with shorter survival.

Objectives: To determine if the magnitude of BACU before treatment is associated with survival in dogs with blastomycosis.

Animals: Fifty-two dogs with blastomycosis.

Methods: Retrospective case review. BACU, radiographic lung severity (RLS) score (0-4 scale), and survival time up to 1 year after diagnosis were obtained through medical record review of dogs with *Blastomyces dermatitidis*.

Results: The overall survival was: discharge, 87%; 1 week, 85%; 2 months, 74%; and 6 months, 69%. BACU correlated with RLS score (r_s = 0.33, P = .02). BACU and RLS scores were lower in survivors to 2 months than nonsurvivors (average BACU difference of 2.5 ng/mL, 95% confidence interval [CI]: 0.2-4.8 ng/mL, P = .04; median RLS difference of 2; range, 0-4, P = .02). Dogs with BACU <5 ng/mL and dogs with mild (0-1) RLS scores had a greater proportion surviving than those with BACU >5 ng/mL (P = .03) and dogs with severe (3-4) RLS scores (P = .04). All dogs with a BACU <5 ng/mL or mild RLS score were alive at last follow-up (median, 365 days; range, 44-365 days). In all, 68.1% of other dogs survived to 2 months (95% CI, 54.8%-84.8%).

Conclusions and Clinical Importance: Dogs with lower BACU and RLS scores have improved survival; however, it is unclear what specific cutoffs should be used for prognosis.

KEYWORDS
canine, prognosis, survival, systemic fungal infection

Abbreviations: BACU, *Blastomyces* antigen concentration in urine; RLS, radiographic lung severity.

1 | INTRODUCTION

Blastomycosis is a systemic fungal infection caused by the fungus *Blastomyces dermatitidis*. The gold standard for the diagnosis of blastomycosis is cytological or histopathological identification of the fungal organism from infected tissue.[1] As tissue collection is not always feasible, safe, or both, and cytologic organism identification can yield false negatives, other diagnostic tests have been developed to aid in diagnosis.

The agar gel immunodiffusion test, which measures antibodies in serum against *B dermatitidis*, is infrequently used as it has poor sensitivity (17%-91%).[1-4] Because of this insensitivity, antigen testing has become the predominant diagnostic of choice for blastomycosis. The main commercial *Blastomyces* antigen test (MVista *Blastomyces* quantitative antigen EIA, MiraVista Diagnostics, Indianapolis, Indiana) for use in dogs is a quantitative enzyme-linked assay (EIA) which detects galactomannan, a component of the fungal cell wall. The sensitivity of the assay when performed on urine ranges from 93% to 100%.[2,5,6] The sensitivity is lower when performed on serum (87%).[2] The test is 95% to 100% specific in healthy dogs and those with nonfungal pulmonary disease. Specificity decreases to 25% in cases of histoplasmosis because of cross-reactivity of the galactomannan antigen detected by the assay.[6]

The *Blastomyces* antigen concentration in urine (BACU) decreases with successful treatment.[5] Therefore, this test is not only useful for diagnosis, but also to monitor therapeutic response and relapse. What is yet unknown is if the BACU provides any prognostic information. Known prognostic indicators of blastomycosis infection include central nervous system involvement, severity of pulmonary disease, and the number of band neutrophils in blood.[1,7-9] In 1 investigation, all dogs requiring mechanical ventilation died.[9] This is logical as the most common cause of death with blastomycosis is respiratory failure.[7,9] Historical cure rates of blastomycosis range from 53% to 75% with 75% of the deaths occurring within the first week of treatment.[7-9] Relapse of disease ranges from 20% to 26%.[5,7]

The primary purpose of this paper was to determine if higher BACU at the time of diagnosis in dogs with confirmed or presumptive blastomycosis is associated with shorter survival. A secondary aim was to determine if a greater radiographic severity of lung involvement is associated with shorter survival and to determine the correlation of radiograph severity with BACU.

2 | MATERIALS AND METHODS

2.1 | Case selection criteria

A retrospective case series was performed. Medical records of dogs presented to the University of Minnesota Veterinary Medical Center between 1 January 2011 and 31 January 2018 were searched to identify dogs in which a BACU test had been submitted to MiraVista Diagnostics. Dogs were eligible for inclusion in the study if the BACU test was performed before antifungal treatment, results were ≥0.2 ng/mL, there was no history of previous episodes of blastomycosis, and their medical records contained adequate information for review. Dogs with a falsely negative BACU test (BACU = 0) before treatment were also included if *Blastomyces* organisms were identified via cytology or histopathology.

Once cases met the inclusion criteria, they were categorized as confirmed *Blastomyces* cases or presumptive. Based on published criteria, cases with cytologic or histologic presence of *Blastomyces* were considered confirmed cases.[9] Cases were considered presumptive if blastomycosis was the primary diagnostic rule out and the BACU was ≥0.2 ng/mL, but there was no histologic or cytologic confirmation of *Blastomyces*.

2.2 | Medical records review

Information on signalment, clinical signs, physical examination findings, BACU at the time of diagnosis and before antifungal treatment, oxygen supplementation requirement, and time to death or last known follow-up (up to 1 year) was obtained from the medical records for each study case.

2.3 | Review of radiographs

Orthogonal radiographic views of the thorax (2- or 3-view study) obtained within 1 week of the BACU before treatment, and before antifungal treatment, were reviewed by 2 of the authors (K.L. Anderson [board certified radiologist] and E.G. Nell [radiology resident in final year of training]) in concert and determined a consensus opinion. Referral radiographs were considered acceptable for review. Pulmonary radiographic findings considered compatible with blastomycosis included unstructured interstitial pattern, structured interstitial pattern (nodules, masses, or both nodules and masses), or alveolar pattern. A combination of the aforementioned pulmonary radiographic findings was also considered compatible with blastomycosis. For each set of radiographs, the number of lung lobes affected (0-7) was recorded. Each set of radiographs was then assigned a radiographic lung severity (RLS) score based on the percentage of the most affected lung lobe, using a modified scoring system which combines published criteria.[8,9] This scoring system predicts survival regardless of lung pattern or number of lobes affected. A score of 0 was given to those radiographs with no pulmonary lesions. Radiographs with greater than 0% but ≤20% involvement of the most affected lung lobe were given a score of 1 and categorized as having mild pulmonary involvement. Those with >20% but ≤40% involvement of the most affected lung lobe were given a score of 2 and categorized as having moderate pulmonary involvement. Those with >40% but ≤60% involvement of the most affected lung lobe were assigned a score of 3 and categorized as having moderate to severe pulmonary involvement. Radiographs with >60% involvement of the most affected lung lobe were assigned a score of 4 and categorized as having severe pulmonary involvement.

2.4 | Sample handling

Urine samples were collected from all dogs before receiving antifungal treatment. The urine was collected via either cystocentesis or midstream free catch. Urine was stored at 4°C for 5 days or less until shipment. All urine samples were shipped on ice, via 2nd day or overnight shipping, to the diagnostic laboratory for blastomycosis antigen testing by EIA (MVista *Blastomyces* quantitative antigen EIA, MiraVista Diagnostics).

2.5 | BACU categorization

Cases were grouped into 3 different categories based on BACU before treatment. Those cases with a BACU before treatment between 0 (false negative) and 4.9 ng/mL were considered to have a low BACU before treatment. Those with a BACU before treatment between 5 and 9.9 ng/mL were considered to have a moderate BACU before treatment. Those with a BACU before treatment between 10 ng/mL and above the limit of quantification (14.7 ng/mL) were considered to have a high BACU before treatment. For those cases with a BACU before treatment above the limit of quantification, their BACU before treatment was recorded as 14.7 ng/mL for statistical analysis.

2.6 | Statistical analysis

Overall survival rates with 95% confidence intervals (CIs) were computed at discharge, 1 week, 2 months, and 6 months after institution of treatment, using the Clopper-Pearson exact method. The proportion of dogs receiving corticosteroids was compared between dogs that did and did not survive until 2 months post-discharge using a Fisher's exact test. To investigate differences in BACU and RLS between dogs that did or did not survive until 2 months post-discharge, *t* tests (for BACU) and Wilcoxon rank-sum tests (for RLS) were performed. The 2 month survival time period was included as the primary outcome analysis because it was most comparable to a previous survival investigation.[10] Additionally, to understand how survival differed by BACU and RLS severity group (as described above), Kaplan-Meier curves were computed for each group and compared with the log-rank test. These statistical analyses were chosen because of the presence of censored survival data, as some dogs were lost to follow-up. Pairwise log-rank tests were also performed, with *P* values adjusted using the Bonferroni-Holm method. The RLS score grouping system used in this study is novel and exploratory. In this dataset, the initial 5 group RLS scoring system resulted in relatively small numbers in half of the groups (<8 dogs each with RLS scores of 0, 1, or 3). To reduce complexity of the system and increase power, this survival analysis was repeated with a simplified categorization of radiographic severity that reduced the number of groups from 5 to 3: mild (RLS scores of 0 and 1), moderate (score of 2), and severe (scores of 3 and 4). Finally, to explore the relationship between BACU and RLS scores, Spearman's correlation was computed. Analyses were performed using R software for statistical computing (R, version 3.6.0. www.r-project.org). *P* values <.05 were considered significant.

3 | RESULTS

3.1 | Study samples

The medical record database search identified 57 dogs that met the initial criteria.

A total of 52 dogs (52 out of 57) met the inclusion criteria and were included in the study. In 42 of the 52 dogs, blastomycosis was confirmed based on the cytologic or histologic identification of *Blastomyces* organisms in any tissue sample. In 10 of the 52 dogs, blastomycosis was considered presumptive. In these dogs, tissue sampling was not performed but the BACU before treatment was positive, and blastomycosis was considered the top differential diagnosis based on signalment and radiographic findings.

Five dogs (5 out of 57) were excluded from the study. One dog was excluded because it previously had a blastomycosis infection. Two dogs were excluded because subsequent BACU tests revealed negative results and other diagnoses were obtained via tissue sampling and at necropsy (neoplasia, heartworm disease). Another dog was excluded because the subsequent BACU was negative, and the radiographic lung changes improved without antifungal treatment. The final case was excluded because although the BACU before treatment was positive (2.7 ng/mL), tissue sampling did not reveal the presence of *Blastomyces*, blastomycosis was not considered the top differential, and there was inadequate follow-up information regarding response to itraconazole.

There were a total of 48 purebred dogs and 4 mixed breed dogs (Supplemental Table S1) included in the study. The most commonly included breeds (>3 dogs) were Labrador Retrievers, Golden Retrievers, German Wirehaired Pointers, and mixed breed dogs. There were 22 castrated males, 18 spayed females, 8 intact males, and 4 intact females. The median age at the time of diagnosis was 5 years (range, 0.6-14 years).

Fifty-one out of 52 (98%) of dogs with blastomycosis had at least 2 clinical signs attributable to blastomycosis. Clinical signs most commonly reported by owners included coughing, anorexia/hyporexia, and lethargy (Supplemental Table S2). The most common physical exam finding in dogs with blastomycosis was fever with 41 out of 52 dogs (79%) having an elevated body temperature at the time of initial examination. Other common physical exam findings included pulmonary auscultation abnormalities (33/52, 63%), cough (32/52, 62%), lymphadenopathy (28/52, 54%), and cutaneous lesions (15/52, 29%; Supplemental Table S3). Twenty-seven percent (14/52) of dogs received oxygen supplementation during hospitalization.

3.2 | Overall survival

Dogs were followed for 1 year. The median follow-up time for dogs alive at the time of last follow-up was 365 days (range, 2-365 days).

TABLE 1 *Blastomyces* antigen concentration in urine (BACU) before treatment category and survival to discharge, 1 week, 2 months, and 6 months

BACU category	Survival to discharge	Survival to 1 week	Survival to 2 months	Survival to 6 months
0-4.99 ng/mL	14/14 (100%)	14/14 (100%)	14/14 (100%)	10/10 (100%)
	95% CI, 77%-100%	95% CI, 77%-100%	95% CI, 77%-100%	95% CI, 77%-100%
5-9.99 ng/mL	19/24 (79%)	18/24 (75%)	13/22 (59%)	12/21 (57%)
	95% CI, 58%-93%	95% CI, 53%-90%	95% CI, 36%-79%	95% CI, 34%-78%
10-4.7 ng/mL	12/14 (86%)	12/14 (86%)	8/11 (73%)	7/11 (64%)
	95% CI, 58%-98%	95% CI, 57%-98%	95% CI, 39%-94%	95% CI, 30%-89%
All	45/52 (87%)	44/52 (85%)	35/47[a] (75%)	29/42[a] (69%)
	95% CI, 74%-94%	95% CI, 72%-93%	95% CI, 60%-86%	95% CI, 53%-82%

[a]Totals for survival at 2 and 6 months are lower than earlier time points because of some dogs being lost to follow-up.

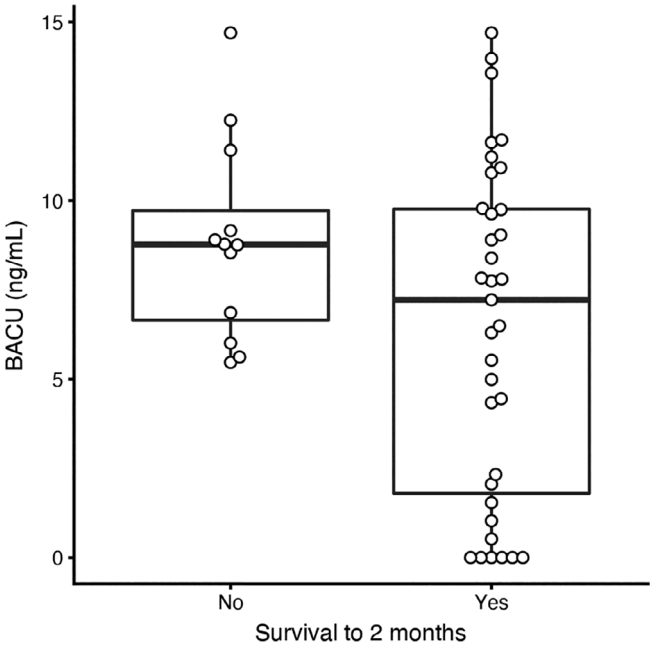

FIGURE 1 Box and whisker plots of *Blastomyces* antigen concentration in urine (BACU) before treatment at the time of diagnosis in dogs that did and did not survive until 2 months post-discharge. The dots represent the BACU before treatment of individual dogs within the specified survival group. The boxes represent the 25th and 75th percentiles. The whiskers extend to the farthest points within 1.5 the interquartile range of the box. The BACU before treatment was significantly different between dogs that did or did not survive until 2 months post-discharge (*t* test average difference = 2.5 ng/mL, 95% confidence interval 0.2-4.8 ng/mL, *t* test *P* = .04)

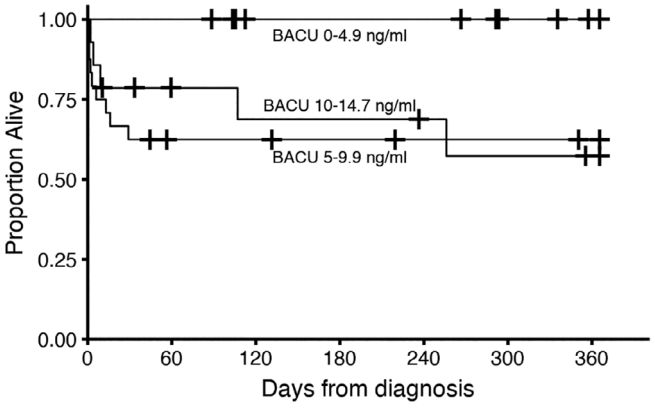

FIGURE 2 Kaplan-Meier survival curve of patient survival grouped by *Blastomyces* antigen concentration in urine (BACU) before treatment. The crosses represent censored individuals at the point of last known follow-up. There were significant differences between BACU before treatment group and overall survival (*P* = .04). Dogs with a BACU before treatment <5 ng/dL lived significantly longer than dogs in the other BACU before treatment groups (*P* = .03 after Bonferroni-Holm correction)

Survival numbers and proportions for each follow-up time point (discharge [range, 1-8 days; average = 2 days], 1 week, 2 months, 6 months) are summarized in Table 1. All 7 dogs that did not survive to discharge died or were euthanized because of respiratory failure (progressive dyspnea/tachypnea, worsening pulse oximetry readings, increasing need for escalation of oxygen support, need for respiratory ventilation) or overall declining clinical status despite antifungal treatment and supportive care. Ten dogs were lost to follow-up between the 1 week and 6 month time point.

For 6 dogs, a known death (natural passing or euthanasia) occurred at some point after discharge and before the 6 month time point. In all 6 of these cases, the decision to euthanize, or natural death, was because of blastomycosis-related complications including progressive inappetence, worsening of respiratory clinical signs, and a general decline in quality of life.

3.3 | Treatment and survival

Forty-six out of 52 dogs (88%) were treated with 1 antifungal medication (microencapsulated itraconazole or fluconazole). Three dogs (6% of total sample) were treated with fluconazole and 42 (81% of total sample) were treated with microencapsulated itraconazole. Six dogs (12% of total sample) received multiple antifungal agents. Two dogs

(4% of total sample) received microencapsulated itraconazole and amphotericin-b, 3 dogs (6% of total sample) received both microencapsulated itraconazole and fluconazole (at different time points), and 1 dog (2% of total sample) received 3 antifungal agents (fluconazole, itraconazole, and amphotericin B). One dog (2% of total sample) was not treated with antifungal medication because of arrest before treatment.

Forty-four out of 52 dogs (85%) received an anti-inflammatory medication during the initial treatment for blastomycosis, whereas 8 (15%) did not receive any anti-inflammatory medications. Of those dogs receiving anti-inflammatory medications, 36 (69% of total sample) received a steroid (prednisone, prednisolone, dexamethasone-sp) and 8 (15% of total sample) received a nonsteroidal anti-inflammatory drug. The proportion of dogs receiving steroids did not differ significantly between dogs that did survive until 2 months post-discharge (22/35, 63%) and dogs that did not survive (10/12, 83%) until 2 months post-discharge (P = .29).

3.4 | BACU before treatment and survival

All of the cases had a BACU test performed at the time of diagnosis, before antifungal treatment. The median BACU before treatment at the time of diagnosis was 7.8 ng/mL (range, 0 to >14.7 ng/mL). The BACU before treatment was significantly different between dogs that did or did not survive until 2 months post-discharge, with results in survivors being an average of 2.5 ng/mL lower than nonsurvivors (95% CI, 0.2-4.8 ng/mL; t test P = .04; Figure 1).

Fourteen dogs (27%) had a BACU before treatment <5 ng/mL. Six of these 14 dogs (12% of total sample) had a false negative BACU before treatment (0 ng/mL). The sensitivity of the BACU test before treatment in this sample was 88% (46/52; 95% CI, 77%-95%). Twenty-four dogs (46%) had a BACU before treatment of 5 to 9.9 ng/mL. Fourteen dogs (27%) had a BACU before treatment of 10 to 14.7 ng/mL (Table 1).

The number of dogs with known status for each time point (discharge, 1 week, 2 months, and 6 months) and proportion surviving are presented in Table 1. All 14 dogs with a BACU before treatment <5 ng/mL survived to the time of last follow-up (median, 323 days; range, 88-365 days); 4 dogs in this group were lost to follow-up between the 2- and 6-month time points. Fifteen of 24 dogs with a BACU before treatment of 5 to 9.9 ng/mL survived to the time of last follow-up (median, 365 days; range, 44-365); 3 dogs in this group were lost to follow-up between the 1 week and 6 month time points. Nine dogs in this group were euthanized or died naturally because of disease from blastomycosis. Ten of 14 dogs with a BACU before treatment of 10 to 14.7 ng/mL survived to the time of last follow-up (median, 306 days; range, 10-365 days); 3 dogs in this group were lost to follow-up between the 1 week and 6 month time points. Four dogs in this group were euthanized or died naturally because of disease from blastomycosis.

The log-rank test comparing the Kaplan-Meier curves for the associations between BACU before treatment categories showed significant differences in survival rates (P = .04; Figure 2). Dogs with a BACU before treatment <5 ng/mL lived significantly longer (P = .03 after Bonferroni-Holm correction) than those with a BACU before treatment in the other categories (Figure 2). There was no significant difference in survival between those dogs with a BACU before treatment of 5 to 9.9 ng/mL compared to those with a BACU before treatment of 10 to 14.7 ng/mL (P = .85 after Bonferroni-Holm correction; Figure 2).

3.5 | RLS score and survival

Fifty (96%) cases had thoracic radiographs performed at the time of diagnosis, within 1 week of the BACU test, and before antifungal treatment. Two dogs did not have radiographs that met the radiograph inclusion criteria. One of these dogs did not have radiographs performed within 1 week of the BACU test before treatment and the other dog only had a single lateral radiograph available for review.

TABLE 2 Radiographic lung severity (RLS) score and survival to discharge, 1 week, 2 months, and 6 months

Radiographic lung severity score	Survival to discharge	Survival to 1 week	Survival to 2 months	Survival to 6 months
0	7/7 (100%)	7/7 (100%)	7/7 (100%)	4/4 (100%)
	95% CI, 59%-100%	95% CI, 59%-100%	95% CI, 59%-100%	95% CI, 40%-100%
1	5/5 (100%)	5/5 (100%)	4/4 (100%)	4/4 (100%)
	95% CI, 48%-100%	95% CI, 48%-100%	95% CI, 40%-100%	95% CI, 48%-100%
2	12/12 (100%)	12/12 (100%)	8/10 (80%)	6/9 (67%)
	95% CI, 74%-100%	95% CI, 74%-100%	95% CI, 44%-98%	95% CI, 30%-93%
3	6/7 (86%)	5/7 (71%)	4/7 (57%)	4/7 (57%)
	95% CI, 42%-100%	95% CI, 29%-96%	95% CI, 18%-90%	95% CI, 18%-90%
4	13/19 (68%)	13/19 (68%)	10/17 (59%)	10/17 (59%)
	95% CI, 43%-87%	95% CI, 43%-87%	95% CI, 33%-82%	95% CI, 73%-82%
All	43/50 (86%)	42/50 (84%)	33/45[a] (73%)	28/41[a] (68%)
	95% CI, 73%-94%	95% CI, 71%-83%	95% CI, 58%-85%	95% CI, 52%-82%

[a]Totals for survival at 2 and 6 months are lower than earlier time points because of some dogs being lost to follow-up.

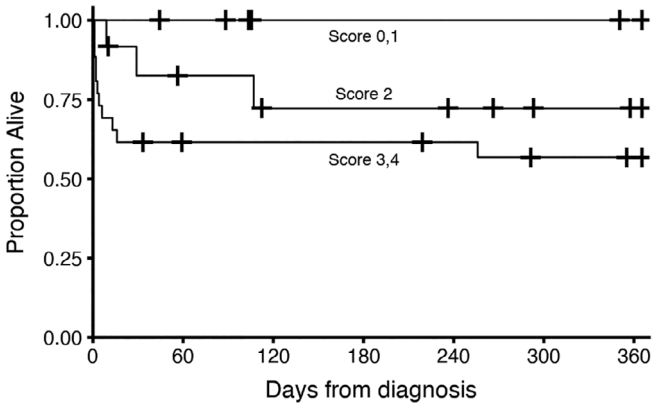

FIGURE 3 Kaplan-Meier survival curve of individuals grouped based on initial radiographic score. There was a significant difference between radiographic lung severity (RLS) score and overall survival time (P = .03) when radiographic categories were combined into three levels (0/1; 2; 3/4). Dogs in the mild RLS category (0/1) lived longer when compared to those in the moderate (2) and severe (3/4) RLS categories (P = .04 after Bonferroni-Holm adjustment)

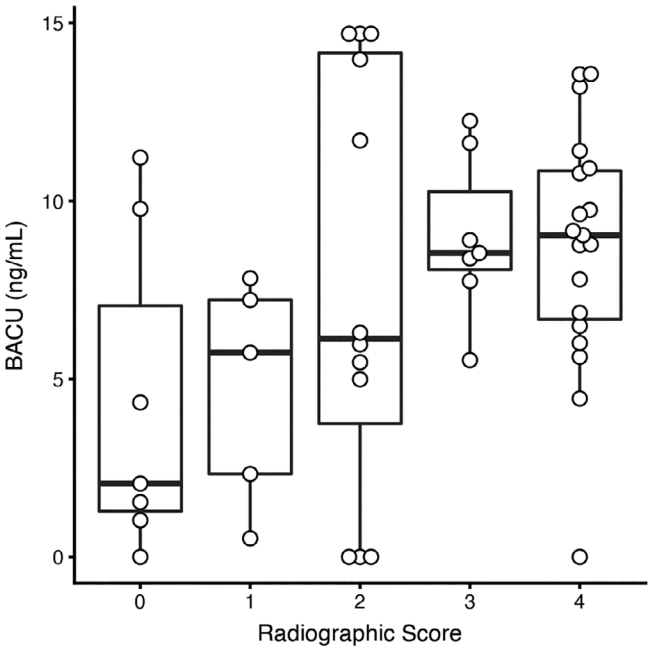

FIGURE 4 Box and whisker plots of *Blastomyces* antigen concentration in urine (BACU) before treatment organized by radiographic lung severity (RLS) score. The dots represent the BACU before treatment of individual dogs within the specified RLS score group. The boxes represent the 25th and 75th percentiles. The whiskers extend to the farthest points within 1.5 the interquartile range of the box. BACU before treatment and RLS score were correlated (r = 0.33; P = .02)

The breakdown of number of lung lobes affected was: 7 dogs (14%) no pulmonary changes, 8 dogs (16%) 1 lobe, 5 dog (10%) 2 lobes, 5 dogs (10%) 3 lobes, 4 dogs (8%) 4 lobes, 3 dogs (6%) 5 lobes, and 18 dogs (36%) 7 lobes. The breakdown of RLS scores assigned was: 7 dogs (14%) score 0, 5 dogs (10%) score 1, 12 dogs (24%) score 2, 7 dogs (14%) score 3, and 19 dogs (38%) score 4 (Table 2).

The number of dogs with known status for each time point (discharge, 1 week, 2 months, and 6 months) and proportion surviving are presented in Table 2. All of the dogs with a RLS score of 0 survived to the time of last follow-up (median, 365; range, 88-365 days); 3 dogs in this group were lost to follow-up between the 2- and 6-month time points. All of the dogs with a RLS score of 1 survived to the time of last follow-up (median, 365; range, 44-365 days); 1 dog in this group was lost to follow-up between the 1-week and 2-month time points. Nine of the dogs with a RLS score of 2 survived to the time of last follow-up (median, 266; range, 10-365 days); 3 of the dogs in the group were lost to follow-up between the 1-week and 6-month time points. Three of the dogs in this group were euthanized because of disease from blastomycosis. Four dogs with a RLS score of 3 survived to the time of last follow-up (median, 311; range, 219-365 days); no dogs were lost to follow-up during the study period. Three of the dogs in this group were euthanized or died naturally because of disease from blastomycosis. Twelve dogs with a RLS score of 4 survived to the time of last follow-up (median, 365; range, 33-365 days); 2 of the dogs in this group were lost to follow-up between the 1-week and 2-month time points. Seven dogs in this group were euthanized or died naturally because of disease from blastomycosis.

The RLS score was higher in dogs that did not survive until 2 months post-discharge than those that did survive until 2 months post-discharge (median difference of 2; Wilcoxon rank-sum P = .02; Table 2). The log-rank test comparing the Kaplan-Meier curves of the 5 RLS categories was not significant (P = .11); however, comparing the simplified radiographic severity categorizations (mild = RLS 0/1, moderate = RLS 2, severe = RLS 3/4) was significant (P = .03; Figure 3), and those in the mild category lived significantly longer than those in the severe category (P = .04 after Bonferroni-Holm adjustment).

3.6 | BACU before treatment and RLS score

The BACU before treatment and RLS score were correlated (r_s = 0.33; P = .02). Median *Blastomyces* antigen concentrations in urine before treatment were lower in dogs with a RLS score of 0 or 1; however, there was overlap among all groups (Figure 4).

4 | DISCUSSION

The results of this study indicate that dogs with blastomycosis and low (<5 ng/mL) *Blastomyces* antigen concentrations in urine before treatment have a higher proportion surviving than those with higher concentrations and longer survival times, although it should be noted that there is great variation in the BACU before treatment (range, 0-14.7 ng/mL) in survivors.

Similar to other studies,[7-9] our results demonstrate that dogs with more severe radiographic pulmonary disease have shorter survival. The RLS scoring system used in this study was modified from previous systems.[8,9] We started with 5 groups (RLS scores 0-5) but also tested a simplified system with 3 groups (mild = 0 or 1, moderate = 2, severe = 3 or 4) because of low numbers of dogs at both ends of the

score range. In the 5 group system, proportion surviving did not differ among groups. However, in the 3 group system, it became apparent that those with a mild RLS score have a higher proportion surviving than those with a severe RLS score.

Based on the results of this study, dogs with lower *Blastomyces* antigen concentrations in urine before treatment and lower initial RLS scores have higher survival rates. However, more than 60% of cases in the moderate to high BACU before treatment groups (≥5 ng/mL), and moderate to severe radiograph category (RLS ≥2), survived to 2 months post-discharge. Because over half of the dogs with a high BACU before treatment survived, BACU before treatment should not be used as a sole prognostic indicator. It is difficult to assess from this current data how much information BACU before treatment adds beyond RLS score or if 1 of the 2 is a better prognostic indicator, as there were too few cases where the BACU before treatment was high and the pulmonary changes were minimal, or vice versa.

Further studies are needed to determine the most clinically relevant cutoffs for BACU before treatment and RLS score before treatment for estimating survival rates. Given the size of the study, it is informative to acknowledge that a BACU before treatment of <5 ng/mL is associated with a greater proportion surviving, but an adequately precise estimate of the survival rate based on BACU before treatment in the clinical setting cannot be determined without a larger, ideally prospective, study. Additionally, further studies are necessary to better understand the survival of subjects with a low BACU before treatment and high initial RLS score or vice versa.

A limitation of this study is that urine concentration (specific gravity) is not considered when measuring the BACU. It is possible that a higher urine specific gravity results in a greater BACU, thereby explaining why in this study many dogs with a high BACU before treatment still had positive outcomes. The sensitivity of the BACU test was comparable to previous reports; however, it is not known if urine-specific gravity influences the sensitivity of the BACU test. Further studies are necessary to determine to what degree urine-specific gravity impacts BACU.

Another limitation is that 19% (10 of 52) cases were lost to follow-up, potentially impacting overall survival results. Additionally, not all cases received the same therapeutic protocol; therefore, we cannot determine to what degree different therapies impacted survival. The proportion of dogs that received corticosteroids was not significantly different between survivors and nonsurvivors, in line with a previous study that found the use of anti-inflammatory medications did not impact survival to 30 days.[10] However, other aspects of treatment could affect outcome. Lastly, because cytologic or histologic identification of *Blastomyces* organisms was not conducted in all cases, it is possible, although unlikely, that the radiographic changes were because of pulmonary disease other than blastomycosis (eg, other infection or neoplasia) and the BACU before the treatment was falsely positive.

In conclusion, dogs with low *Blastomyces* antigen concentrations in urine before treatment (<5 ng/mL) survived longer than those with higher concentrations. Survival curves were not significantly different between RLS scores before treatment when a 5 group system (score 0-4) was used but were when the scoring system was simplified to 3 severity groups. In the 3 group system, dogs with a mild RLS score survived longer.

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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

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Crews LJ, Feeney DA, Jessen CR, Newman AB, Sharkey LC. Utility of diagnostic tests for and medical treatment of pulmonary blastomycosis in dogs: 125 cases (1989–2006). *J Am Vet Med Assoc*. 2008;232(2):222-227. https://doi.org/10.2460/javma.232.2.222.
2. Spector D, Legendre AM, Wheat J, et al. Antigen and antibody testing for the diagnosis of blastomycosis in dogs. *J Vet Intern Med*. 2008;22(4):839-843. https://doi.org/10.1111/j.1939-1676.2008.0107.x.
3. Klein BS, Squires RA, Lloyd JKF, Ruge DR, Legendre AM. Canine antibody response to *Blastomyces dermatitidis* WI-1 antigen. *Am J Vet Res*. 2000;61(5):554-558. https://doi.org/10.2460/ajvr.2000.61.554.
4. Legendre AM, Becker PU. Evaluation of the agar-gel immunodiffusion test in the diagnosis of canine blastomycosis. *Am J Vet Res*. 1980;41(12):2109-2111.
5. Foy DS, Trepanier LA, Kirsch EJ, Wheat LJ. Serum and urine *Blastomyces* antigen concentrations as markers of clinical remission in dogs treated for systemic blastomycosis. *J Vet Intern Med*. 2014;28(2):305-310. https://doi.org/10.1111/jvim.12306.
6. Mourning AC, Patterson EE, Kirsch EJ, et al. Evaluation of an enzyme immunoassay for antibodies to a recombinant *Blastomyces* adhesin-1 repeat antigen as an aid in the diagnosis of blastomycosis in dogs. *J Am Vet Med Assoc*. 2015;247(10):1133-1138. https://doi.org/10.2460/javma.247.10.1133.
7. Legendre AM, Rohrbach BW, Toal RL, Rinaldi MG, Grace LL, Jones JB. Treatment of blastomycosis with itraconazole in 112 dogs. *J Vet Intern Med*. 1996;10(6):365-371. https://doi.org/10.1111/j.1939-1676.1996.tb02082.x.
8. Legendre AM, Selcer BA, Edwards DF, Stevens R. Treatment of canine blastomycosis with amphotericin B and ketoconazole. *J Am Vet Med Assoc*. 1984;184(10):1249-1254.
9. Crews LJ, Feeney DA, Jessen CR, Newman AB. Radiographic findings in dogs with pulmonary blastomycosis: 125 cases (1989–2006). *J Am Vet Med Assoc*. 2008;232(2):215-221. https://doi.org/10.2460/javma.232.2.215.
10. Walton RAL, Wey A, Hall KE. A retrospective study of anti-inflammatory use in dogs with pulmonary blastomycosis: 139 cases

(2002–2012). *J Vet Emerg Crit Care*. 2017;27(4):439-443. https://doi.org/10.1111/vec.12615.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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