Serum C-reactive protein and haptoglobin decrease in the first three months of treatment and relative change in haptoglobin predict remission in dogs with pulmonary coccidioidomycosis

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OBJECTIVE
To evaluate temporal changes in serum C-reactive protein (CRP) and haptoglobin (Hp) concentrations in dogs with pulmonary coccidioidomycosis and assess their utility to detect remission.

METHODS
31 client-owned dogs with newly diagnosed pulmonary coccidioidomycosis from October 2020 to February 2021 were included in a retrospective cohort study that utilized archived serum. Serum was originally obtained at diagnosis and once every 3 months after antifungal administration until either remission or 12 months. Time points were designated as baseline (T0), 3 months (T1), 6 months (T2), 9 months (T3), and 12 months (T4). Serum CRP and Hp were measured at a reference laboratory with ELISA assays.

RESULTS
Median serum CRP and Hp concentrations decreased from T0 (CRP, 56 mg/L; Hp, 716.1 mg/dL) to T1 (CRP, 3.3 mg/L; Hp, 240.5 mg/dL); subsequent decreases were not significant. Eighteen (60%) and 16 (53%) of 30 dogs had normal serum CRP and Hp concentrations at T1, respectively. Absolute serum CRP (AUC, 0.58; 95% CI, 0.45 to 0.72) and Hp (AUC, 0.65; 95% CI, 0.52 to 0.78) were poor detectors of remission. However, the percentage change in Hp from T0 to T1 (AUC, 0.90; 95% CI, 0.74 to 1.0) was an excellent predictor of remission within 12 months.

CONCLUSIONS
Serum CRP and Hp concentrations decrease in the first 3 months of antifungal treatment in dogs with pulmonary coccidioidomycosis, and the percentage change of Hp may help predict dogs that will achieve remission within 12 months of treatment.

CLINICAL RELEVANCE
Serum CRP and Hp may be useful adjunctive biomarkers to monitor treatment response in dogs with pulmonary coccidioidomycosis.

Keywords: Valley fever, Coccidioides, fluconazole, acute phase protein, cough

Coccidioidomycosis, or Valley fever, is caused by inhalation of soil-dwelling dimorphic fungi with an expanding region of endemicity but concentrated most densely in the southwestern US. Pulmonary coccidioidomycosis is the most common form of infection in dogs with clinical signs that include one or more of cough, exercise intolerance, wheezing, respiratory difficulty, or tachypnea, along with nonspecific signs such as lethargy, decreased appetite, vomiting, weight loss, or diarrhea.1-4

First-line antifungal treatment options for clinically stable dogs with pulmonary coccidioidomycosis include fluconazole or itraconazole. 5 Currently, there is a void in evidence-based recommendations for treatment monitoring and criteria to reliably detect remission so that antifungal treatment can be safely discontinued. At present, guidelines are based on anecdotal expert opinion. 1,5 The lack of structured treatment-monitoring guidelines has likely contributed to prolonged administration of antifungal drugs in some dogs with pulmonary coccidioidomycosis and, with it, increased risk for antifungal-related complications and financial burden.
Positive acute-phase proteins (APPs) including C-reactive protein (CRP) and haptoglobin (Hp) are produced as a reaction to various types of systemic inflammation. Positive APP can be categorized as being major or moderate based on the rapidity with which it peaks and decreases after exposure and subsequent resolution of inflammation as well as the magnitude of peak elevation. A recent veterinary editorial publication recommends incorporating a major and moderate positive APP in diagnostic plans for companion animals. In dogs, CRP (major) and Hp (moderate) are typically the most common combination of positive APP utilized. Temporal changes in blood positive APP concentrations have been used in humans and dogs with various forms of infections to guide decisions to discontinue antimicrobial treatment but have not been investigated in coccidioidomycosis.

The objectives of this study were to characterize temporal changes in serum CRP and Hp concentrations in dogs with newly diagnosed pulmonary coccidioidomycosis after initiation of antifungal treatment and determine whether serum CRP and Hp concentrations were useful at detecting remission. We hypothesized that a significant decrease in serum CRP and Hp concentrations would occur within the first 6 months of antifungal administration and that both biomarkers would be reliable predictors of remission.

Methods

Criteria for selection of cases

Client-owned dogs with a new diagnosis of pulmonary coccidioidomycosis between October 2020 and February 2021 were eligible for inclusion. Dogs included in this study were used primarily in a separate study aimed at investigating a novel treatment-monitoring protocol for dogs with pulmonary coccidioidomycosis. This same cohort of dogs was also used in a recently published study that evaluated tracheobronchial lymphadenopathy in dogs with pulmonary coccidioidomycosis. The current study, from which the archived samples originated, was conducted in accordance with guidelines for clinical studies and approved by the Midwestern University Animal Care and Use Committee (protocol No. 3024). Written informed consent was obtained for all included dogs that had archived serum.

Dogs were required to exhibit 1 or more clinical sign associated with respiratory tract disease, including cough, wheeze, increased respiratory effort, exercise intolerance, tachypnea, cyanosis, or syncope, along with a positive anti-Coccidioides spp antibody serologic test result, and had a minimum of 2-view thoracic radiographs available for review. Dogs with unremarkable thoracic radiographs at the time of diagnosis were still eligible for inclusion if there was a strong clinical suspicion for pulmonary coccidioidomycosis in conjunction with adequate improvement in clinical signs and antibody serologic test results after initiation of antifungal treatment. A single reference laboratory (MiraVista Diagnostics) was used for serological testing for IgM and IgG against Coccidioides spp using agar gel immunodiffusion (AGID; IgM and IgG) and enzyme immunoassay (EIA; IgG) at baseline and at all subsequent evaluations. Positive AGID IgM or IgG was defined as detectable antibodies in an undiluted serum sample. If positive, serial dilutions were tested (up to 1:128), and the highest dilution with detectable IgG antibodies was reported as the final serum titer result. A positive EIA IgG was defined as results that were ≥ 10 EIA units (EU), and the upper quantifiable range was 80 EU. Exclusion criteria included disseminated disease, comorbid disorders with potential immune dysregulation, administration of immunomodulating medications, and initiation of antifungal treatment for > 7 days before enrollment. Dogs were also excluded if there was insufficient volume of residual serum to measure CRP and Hp concentrations.

Study design

A retrospective cohort study was performed utilizing archived serum samples. Dogs were presented for evaluation once every 3 months after initiation of an FDA-approved generic formulation of fluconazole until remission was accomplished or for a maximum of 12 months. These time points are designated as baseline (ie, time of diagnosis; T0), 3 months (T1), 6 months (T2), 9 months (T3), and 12 months (T4). Three-view thoracic radiographs and antibody serology were performed at each evaluation. Clinical status (ie, subclinical or clinical) was determined at each visit based on the presence or absence of signs related to respiratory tract disease. The attending clinician, not the research investigators, made all other diagnostic and treatment decisions. Remission was defined as the first evaluation at which all 3 of the following criteria were met: (1) anti-Coccidioides IgG titer of ≤ 1.8, (2) thoracic radiographs that were either unremarkable or static mild disease without tracheobronchial lymphadenopathy compared to previous evaluation, and (3) dogs were subclinical. Antifungal administration was discontinued at the time remission was achieved.

Sample and data collection

Medical records were reviewed for each dog enrolled, and age, sex, weight, and breed were recorded. Residual serum from each dog procured at baseline and all subsequent evaluations was stored at −80 °C for a maximum of 2 years before batch analysis of CRP and Hp was performed. Serum CRP and Hp measurements were performed by VDI Laboratory (Chatsworth, CA) as previously described. Reference intervals for the CRP assay in dogs are as follows: normal (≤ 3.9 mg/L), mildly increased (4 to 9.9 mg/L), moderately increased (10 to 39.9 mg/L), and markedly increased (≥ 40 mg/L). The upper limit of the reference interval (ie, increased) for Hp assay is 250 mg/dL. The lower quantifiable limit for serum CRP is 0.5 mg/L and for Hp is 10 mg/dL.

Statistical analysis

Statistical analyses were conducted with SigmaPlot (Systat Software) and Stata Statistical Software,
version 18 (StataCorp LLC). Nonnormally distributed continuous data were presented as median and IQR and were expressed as quartile 1 and quartile 3, as well as range when indicated. Normally distributed data was presented as mean and standard deviation (SD). Categorical data were presented as proportions. Percentage change in serum CRP and Hp from T0 to T1 was calculated with the following formula: \((T0 – T1)/T0 \times 100\). Spearman correlation coefficient was used to assess the strength of association between the percentage change of serum CRP and Hp from T0 to T1. The strength level of the relationship between variables was defined with the following Spearman \(p\) intervals: weak (0.1 to 0.39), moderate (0.4 to 0.69), strong (0.7 to 0.89), and very strong (0.9 to 1.0). Receiver operating characteristic (ROC) analysis was used to determine the utility of CRP and Hp for predicting remission at each time point and the percent change in CRP and Hp at the 3-month time point for predicting remission within the remaining study period. The optimal cut points for the ROC analyses were determined via the Liu method. The area under the curve (AUC) was interpreted as perfect (1.0), excellent (0.9 to 0.99), good (0.8 to 0.89), fair (0.7 to 0.79), poor (0.51 to 0.69), and no value (0.5). \(P < .05\) was the cutoff for significance.

## Results

### Dogs

Thirty-two dogs were eligible for inclusion, but 1 dog was excluded because of insufficient residual serum from all evaluation time points that were needed for CRP and Hp measurements, leaving 31 dogs included in this study. Information regarding the number of dogs evaluated at each time point, proportion with residual serum available, and number of dogs achieving remission can be found in Figure 1. The median age was 4.8 years (IQR, 1.7 to 8.0), and the mean weight was 22.9 kg (SD, 13.3). There were 18 purebred dogs and 13 mixed-breed dogs. Purebred dogs included Chihuahua (n = 3), Rhodesian Ridgeback (3), American Pit Bull Terrier (n = 2), and 1 each of Australian Cattle Dog, American Foxhound, Belgian Malinois, English Bulldog, English Mastiff, German Shorthaired Pointer, Golden Retriever, Labrador Retriever, Miniature Poodle, and Queensland Heeler. The sex distribution was as follows: castrated male (12/31 [39%]), spayed female (12/31 [39%]), intact female (4/31 [13%]), and intact male (3/31 [9%]).

### Clinical Information

The most common clinical signs at diagnosis included cough (31/31 [100%]), decreased appetite (14/31 [45%]), lethargy (14/31 [45%]), exercise intolerance (5/31 [16%]), tachypnea (4/31 [13%]), diarrhea (4/31 [13%]), wheezing (4/31 [13%]), increased respiratory effort (2/31 [6%]), sneeze (2/31 [6%]), vomiting (2/31 [6%]), and weight loss (1/31 [3%]). The mean respiratory rate at diagnosis was 35.7 breaths/min (SD, 8.5). The mean rectal temperature was 103.3 °F (SD, 1.1; n = 28). The median duration of time clinical signs were observed by owners before evaluation was 14 days (IQR, 7 to 37). Twenty-nine percent (9/31) of dogs had positive detection of anti–*Coccidioides* spp IgM antibodies. Ninety-four percent (29/31) of dogs had positive detection of IgG antibodies via AGID, and the median titer was 1:16 (range, 1:2 to 1:128). Sixty-five percent (20/31) of dogs had positive detection of IgG antibodies via EIA, and the mean value was 44.1 EU (SD, 21.9). Thoracic radiographic description information for this cohort has been previously reported.2 Dogs were treated with an FDA-approved generic formulation of fluconazole at a median PO dosage of 15.9 mg/kg/d (IQR, 13.6 to 18.6). One dog was switched to amphotericin B lipid complex (Abelcet; Lediant Biosciences) and itraconazole (Sporonox; Janssen Pharmaceuticals) at the 3-month evaluation (day 93) because of refractory disease. Twenty-six percent (8/31) of dogs were administered prednisone at a mean PO dosage of 0.7 mg/kg/d (SD, 0.27) for a mean duration of 11.9 days (SD, 3.4).

### Baseline serum CRP and Hp

Ninety-seven percent (28/29) of dogs that had adequate residual serum available at T0 (ie, baseline) had increased CRP concentrations, and the median result was 55.7 mg/L (IQR, 47.6 to 67.2). Most dogs were categorized as having marked increases in CRP concentrations (24/29 [83%]; Table 1). Ninety-three percent (27/29) of dogs with residual serum at T0 had increased Hp concentrations, and the median result...
was 716.1 mg/dL (IQR, 651.6 to 744.7). The dog with a normal CRP concentration also had a normal Hp result. There were several clinical aspects from this case that reinforced our clinical diagnosis of pulmonary coccidioidomycosis. While this dog had unremarkable thoracic radiographs, the dog had detection of IgM antibodies at T1 (ie, 3-month evaluation), eventually became seronegative (baseline IgG titer of 1:4), and had substantial clinical improvement with fluconazole administration. The other dog with a normal Hp concentration had a mild increase in CRP. This dog also had unremarkable thoracic radiographs at diagnosis but had improvement in IgG titers (baseline of 1:8 and decreased to 1:1) and resolution of clinical signs after administration of fluconazole.

Temporal assessment of serum CRP and Hp

Twenty-eight dogs had sufficient serum to measure CRP and Hp concentrations at both T0 and T1. Serum CRP and Hp concentrations decreased in 89% (25/28) and 93% (26/28), respectively, of dogs from T0 to T1. Three dogs had mild increases in serum CRP concentrations. Likewise, 2 different dogs had mild increases in serum Hp concentrations (Supplementary Table S1). The median percentage improvement from T0 to T1 for serum CRP and Hp was 92% (IQR, 57 to 97) and 39% (IQR, 14 to 83), respectively. There was a moderate positive association between the percentage change in serum CRP and Hp from T0 to T1 (p = 0.53; P = .004).

Table 1—Descriptive summary of categorical classification of serum C-reactive protein and haptoglobin results in dogs with newly diagnosed pulmonary coccidioidomycosis before (baseline; T0) and 3 months (T1), 6 months (T2), 9 months (T3), and 12 months (T4) after initiation of PO antifungal administration.

<table>
<thead>
<tr>
<th></th>
<th>T0 (n = 29)</th>
<th>T1 (n = 30)</th>
<th>T2 (n = 24)</th>
<th>T3 (n = 19)</th>
<th>T4 (n = 9)</th>
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<tr>
<td>C-reactive protein</td>
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<td>Normal (%)</td>
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<td>18 (60)</td>
<td>15 (63)</td>
<td>12 (63)</td>
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<td>2 (7)</td>
<td>2 (8)</td>
<td>3 (16)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>Moderate increase (%)</td>
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<td>6 (20)</td>
<td>4 (17)</td>
<td>3 (16)</td>
<td>0 (0)</td>
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<tr>
<td>Marked increase (%)</td>
<td>24 (83)</td>
<td>4 (13)</td>
<td>3 (12)</td>
<td>1 (5)</td>
<td>0 (0)</td>
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<td>Haptoglobin</td>
<td></td>
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<tr>
<td>Increased (%)</td>
<td>27 (93)</td>
<td>14 (47)</td>
<td>14 (58)</td>
<td>8 (42)</td>
<td>6 (67)</td>
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n = Number of dogs with serum sample available.

Figure 2—Serum CRP (A) and haptoglobin (B) concentrations over each time period overlaid by a Lowess curve. The dashed horizontal lines represent the upper limit of normal (3.9 mg/L [A] and 250 mg/dL [B]). Time points included T0 (n = 29), T1 (30), T2 (24), T3 (19), and T4 (9).
of dogs with increased serum Hp concentrations at T0 had normal results by T1. A descriptive summary of data for temporal categorical classification of CRP and Hp can be found in Table 1. Individual dog data can be found in Supplementary Table S1.

Descriptive assessment of remission

Eighty-four percent (26/31) of dogs achieved remission in a mean of 258.9 days (SD, 108.9). Ninety-two percent (24/26) of dogs had sufficient serum at the time of remission to quantify CRP and Hp concentrations. The median serum CRP and Hp concentrations at remission were 2.6 mg/L (IQR, 1.1 to 6.6) and 214 mg/dL (IQR, 44.8 to 383.3), respectively. Sixty-three percent (15/24) of dogs had serum CRP results that were normal, and the remaining dogs were classified as mild (5/24 [21%]), moderate (5/24 [13%]), and markedly increased (1/24 [4%]) at remission. Sixty-three percent (15/24) of dogs had normal Hp results at remission. The 1 dog with a marked increase in serum CRP and elevated Hp concentrations had been recently diagnosed with skin allergies and self-inflicted wounds to both forelimbs and paws that were addressed the day of remission. Alternative causes for elevations in serum CRP and Hp concentrations in the remaining dogs at remission are unknown.

Sixty percent (3/5) of the dogs that had an increase in either serum CRP or Hp concentrations from T0 to T1 failed to achieve remission in the study period. The remaining 2 dogs eventually achieved remission but experienced more severe disease that required adjusting antifungal therapy or needed a long duration of treatment. One dog achieved remission at T3, but this patient was switched to amphotericin B and itraconazole after refractory disease became a concern at T1. The other dog remained being treated with fluconazole and attained remission at T4. Supplementary Figure S1 illustrates temporal changes in serum CRP and Hp for the 5 dogs that had an increase in either CRP or Hp from T0 to T1.

We were interested in determining whether differences in CRP or Hp existed between dogs that did and did not attain remission in the study period. Therefore, comparisons of absolute CRP and Hp concentrations were made between these subgroups at T0 and T1. The percent change in these APPs between T0 and T1 were also compared between dogs that did and did not achieve remission. There was no difference in serum CRP ($P = .81$) or Hp ($P = .45$) at T0 in dogs that did (CRP median, 55.7 mg/L; CRP IQR, 52.6 to 61.8; Hp median, 716.1 mg/dL; Hp IQR, 675.2 to 741.6; n = 23) and did not (CRP median, 67.9 mg/L; CRP IQR, 6.9 to 71.3; Hp median, 742.3 mg/dL; Hp IQR, 454.6 to 754.2; 5) attain remission. Similarly, no differences in serum CRP ($P = .86$) or Hp ($P = .05$) concentrations were identified at T1 between dogs that achieved remission (CRP median, 3.5 mg/L; CRP IQR, 1.6 to 17.8; Hp median, 232.4; Hp IQR, 100.4 to 549.6; n = 23) and those that did not (CRP median, 3.2 mg/L; CRP IQR, 2.0 to 12.7; Hp median, 575.5 mg/dL; Hp IQR, 354.2 to 753.9; 5). Dogs that achieved remission had a greater percent decrease in Hp from T0 to T1 (median, 63.3%; IQR, 19.5 to 84.5; n = 23) than dogs that did not attain remission (median, 2.3%; IQR, −1.1 to 25.8; 5; $P = .006$). No differences in percent change from T0 to T1 were identified for CRP between dogs that achieved remission (median, 93.6%; IQR, 68.1 to 96.8; n = 23) and those that did not (median, 73%; IQR, −23.4 to 97.1; 5; $P = .53$). A descriptive illustration of temporal changes in serum CRP and Hp concentrations for the 5 dogs that did not attain remission can be found in Figure 3.

Figure 3—Spaghetti plot illustrating temporal changes in serum CRP (A) and haptoglobin (B) in 5 dogs with pulmonary coccidioidomycosis that did not attain remission within 12 months of PO antifungal medication administration. The solid black horizontal line represents the median. The dashed horizontal lines represent the upper limit of normal (3.9 mg/L [A] and 250 mg/dL [B]). The enclosed circles represent individual dog data.
Prediction of remission status

First, absolute serum concentrations of CRP and Hp were evaluated for their utility in identifying remission status. As a test for persistence of clinical disease (ie, absence of remission), serum CRP concentration at its optimum cutoff of 2.5 mg/L had a sensitivity of 61% (95% CI, 48% to 74%) and specificity of 50% (95% CI, 29% to 71%), and Hp concentration at a cutoff of 259 mg/dL had a sensitivity of 53% (95% CI, 39% to 66%) and specificity of 71% (95% CI, 49% to 87%; Figure 4). The AUC for the ROC curve was 0.58 (95% CI, 0.45 to 0.72) and 0.65 (95% CI, 0.52 to 0.78) for serum CRP and Hp concentrations, respectively (Supplementary Table S2).

Next, we wanted to assess whether the percent change in serum CRP or Hp concentration within the first 3 months of antifungal treatment was predictive of achieving remission within 12 months (Supplementary Table S3). For percent change in CRP, the optimum cutoff value was 75%, which resulted in a sensitivity of 70% (95% CI, 47% to 87%) and specificity of 60% (95% CI, 15% to 95%). The optimum cutoff for percent change in Hp was 17%, which conveyed a sensitivity of 83% (95% CI, 61% to 95%) and specificity of 80% (95% CI, 28% to 99%; Figure 5). The AUC for the ROC curve was 0.59 (95% CI, 0.26 to 0.92) and 0.90 (95% CI, 0.74 to 1.0) for CRP and Hp, respectively.

Figure 4—Receiver operating characteristic curve for detection of persistence in clinical disease (ie, absence of remission) and the value of CRP (A) and haptoglobin (B). Optimal cutoff is indicated by a green circle. The black line is reference.

Figure 5—Receiver operating characteristic curves for percent change between T0 and T1 and remission by T4 for CRP (A) and haptoglobin (B), based on dogs that had data from both T0 and T1 (n = 28). Optimal cutoff is indicated by a green circle. The black line is reference.
Discussion

The measurement of APP is commonly used in humans to assess treatment response and guide clinical decisions and has become more common in dogs with various types of infections. Currently, there are no evidence-based guidelines for treatment monitoring in dogs with pulmonary coccidioidomycosis, and thus, incorporation of an objective biomarker into the decision-making algorithm has potential value. Therefore, the objectives of this study were to describe longitudinal changes in serum CRP and Hp concentrations in dogs with pulmonary coccidioidomycosis from diagnosis until remission or a maximum of 12 months and whether these biomarkers can accurately detect remission status. All but 1 dog had increased serum CRP concentrations at T0, and more than 80% had markedly increased results. Sixty percent (18/30) of dogs had normal serum CRP concentrations at T1, and this included approximately half of the dogs with marked increases at T0. Similarly, all but 2 dogs had increased Hp concentrations at T0, and nearly half had normalization at T1. Serum CRP and Hp concentrations decreased precipitously after the first 3 months of antifungal treatment, with gradual decreases thereafter. Absolute serum CRP and Hp concentrations proved to be poor predictors of remission status in our cohort of dogs. However, the percent change in Hp from T0 to T1 was an excellent predictor of achieving remission within 12 months.

Ninety-seven percent (28/29) of dogs had an increased serum CRP and/or Hp concentration at the time of diagnosis, and most dogs (24/29 [83%]) had markedly increased CRP levels according to the laboratory-derived severity scale. The acute-phase response in coccidioidomycosis includes an initial influx of neutrophils with subsequent contribution by macrophages into lung tissue to mediate pathogen clearance. This initial immune response is associated with a robust increase in proinflammatory cytokine expression and tissue damage that is expected to induce production of positive APPs such as CRP and Hp. Serum CRP concentrations are commonly increased in human patients with pulmonary coccidioidomycosis. Likewise, serum CRP concentrations have been shown to be higher in dogs with pulmonary coccidioidomycosis than healthy controls. Little information exists regarding serum Hp concentrations in Coccidioides spp. infections, with the literature comprised of only a study in rats that demonstrated increased serum Hp levels with experimental infection. Collectively, the large proportion of dogs with increased serum CRP and Hp concentrations and the magnitude of elevation reinforce the potential these biomarkers have for prognostication and treatment monitoring in dogs with pulmonary coccidioidomycosis that warrants additional investigation.

There was a significant decrease in both serum CRP and Hp concentrations from T0 to T1, with continued mild improvements thereafter. Serum CRP and Hp decreased from T0 to T1 in most dogs, with median percentage improvement of 92% and 39%, respectively. These results support our hypothesis and have standalone value in regard to providing novel insight into treatment response in dogs with pulmonary coccidioidomycosis. Spherule rupture triggers acute inflammatory responses with downstream effects that perpetuate inflammation and tissue damage. Therefore, it is reasonable to suspect that serum CRP and Hp concentrations may reflect the magnitude of ongoing spherule rupture and propagation much like serum CRP concentrations remaining elevated in dogs with trypanosomiasis and leishmaniasis that have persistence of organisms during treatment. This would suggest that most dogs with pulmonary coccidioidomycosis have minimal fungal growth after 3 to 6 months and may not benefit from continued PO azole administration. Interestingly, the most recent Infectious Diseases Society of America clinical practice guideline for the treatment of coccidioidomycosis recommends that people with uncomplicated pulmonary coccidioidomycosis be treated for 3 to 6 months or longer, depending on the clinical response.

A lack of expected improvement in serum CRP and/or Hp concentrations within 3 to 6 months of treatment in conjunction with clinical signs, physical examination, and thoracic radiographs may prompt reassessment of the antifungal regimen (ie, dose or type). The persistently marked elevation in serum CRP concentration at T1 in 1 dog coincided with the clinician’s concern for refractory disease that triggered a change in antifungal treatment. The serum CRP concentration substantially improved after this change, and the dog eventually went into remission at T3. Another dog with minimal early improvement in serum CRP concentration ultimately went into remission at T4, and 3 others with worsened CRP or Hp did not attain remission in the study period. It is unknown whether incorporation of measuring these serum APPs into the diagnostic follow-up plan and their subsequent call for modification in antifungal treatment would have changed the outcome in the aforementioned dogs. Future clinical trials including serum CRP and Hp concentrations into therapeutic decision-making algorithms are needed to fully determine their value in dogs with pulmonary coccidioidomycosis.

Absolute values of serum CRP and Hp concentrations proved to be poor diagnostic tests to detect remission status in dogs with pulmonary coccidioidomycosis in our study. These results must be interpreted with caution because their performance was directly linked to the criteria used to define remission in our study. Specifically, remission was defined as the first evaluation after initiation of antifungal treatment in which the dog was completely subclinical with IgG titers ≤1:8 and thoracic radiographs that were either unremarkable or had mild static changes without tracheobronchial lymphadenopathy. This constellation of required criteria was intentionally strict in order to avoid declaring remission too early but likely resulted in protracted treatment times and may have skewed interpretation of diagnostic performance of serum CRP and Hp.

While absolute values for serum CRP and Hp were poor predictors of remission status in our study,
the percent change of Hp from T0 (ie, baseline) to T1 (3 months of treatment) was an excellent predictor that a dog would achieve remission within 12 months of antifungal treatment. In contrast, the percent change in CRP was poorly predictive of eventual remission within the study period. The reason the percent change in Hp, but not CRP, during the first 3 months of treatment was useful at predicting remission within 12 months is unknown and is beyond the scope of this study. However, there are several possible theories that independently or in tandem may explain this finding. The lung has been identified as a major site of extrahepatic synthesis of Hp in mice, people, and baboons.22,23 To the authors’ knowledge, expression of Hp gene in the lung of dogs has not yet been investigated. However, if lung tissue production of Hp in dogs parallels other species, then a greater proportional decrease early in the course of antifungal treatment may occur in dogs with a more robust improvement in lung inflammation and/or damage. Another possible explanation is that cortisol (exogenous and endogenous) stimulates Hp production as occurs with alkaline phosphatase, but not CRP, in dogs.27–30 Therefore, it is possible that in dogs, persistent systemic involvement from pulmonary coccidioidomycosis resulted in increased endogenous cortisol production and thus, higher Hp concentrations at T1. This would translate to a lower proportional decrease of Hp in more severely affected dogs. Likewise, some dogs were administered short courses of prednisone after diagnosis, which may have increased Hp concentrations at T1. Generally, prednisone is administered to more severely affected dogs with pulmonary coccidioidomycosis to temporarily ameliorate clinical signs and bridge the gap until improvement is achieved with antifungal treatment. Consequently, dogs receiving prednisone may have been more severely affected with longer-term implications (ie, delayed remission).

Our study had several limitations that must be considered. Dogs were diagnosed with pulmonary coccidioidomycosis based on interpretation of clinical signs, physical examination, clinicopathologic results, thoracic imaging, and anti-Coccidioides spp antibody levels. The use of clinical criteria rather than identification of fungal organisms is more practical in everyday practice because it does not require expensive and invasive diagnostic tests that are not usually pursued in dogs with the pulmonary form of disease. The motivation to pursue this study came after completion of the primary study evaluating a novel treatment-monitoring protocol in dogs with pulmonary coccidioidomycosis. As such, we were dependent on the availability of residual serum from dogs in that study, and this contributed to a lack of APP measurements from 6% (8/119) of available time points. Another limitation is that 26% (8/31) were administered anti-inflammatory doses (median, 0.7 mg/kg/d) of prednisone for a median of 11.9 days after diagnosis. It is possible that the short duration of prednisone administration may have affected serum Hp concentrations at T1 and contributed to the discordant percentage improvement between CRP (median, 92%) and Hp (median, 39%). Our study focused only on serum CRP and Hp because these appear to be the most commonly utilized APPs in dogs. With that said, other APPs such as serum amyloid A and α-1-acid glycoprotein may have been useful and warrant future consideration. The small sample size limited the precision of sensitivity and specificity for predicting remission based on CRP and Hp. Our criteria for remission may have affected statistical testing aimed at evaluating the diagnostic performance of serum CRP and Hp to detect remission. As mentioned earlier, this was unavoidable given the dependence on residual serum from the primary study that had different objectives. A representative example that highlights this limitation is a dog that failed to achieve remission predominately because the owner reported a very mild and intermittent cough a few times per week, which was the only factor that prevented a classification of remission. At T2, that dog had unremarkable thoracic radiographs and normal or mildly elevated APPs (CRP, 1.5 mg/L; Hp, 302.9 mg/dL); the dog’s anti-Coccidioides spp antibody results were an IgM of negative and an IgG of 1:1 (undiluted). Future studies evaluating diagnostic performance of serum CRP and Hp may benefit from more relaxed criteria for remission. Lastly, the normal reference interval and intervals used to categorize severity of CRP elevations were established by the reference laboratory used in this study. Other commercial reference veterinary laboratories and publications commonly use an upper limit of 10 mg/L as normal for dogs.31 Therefore, all the dogs classified as having mildly elevated CRP in this study would often be considered as normal if measured by some other reference laboratories.

In conclusion, our study provides data on the temporal patterns of serum CRP and Hp in dogs with pulmonary coccidioidomycosis before and after antifungal treatment. These data may be useful for clinicians using biomarkers to monitor treatment response and for designing clinical trials. Results of our study suggest that most dogs with pulmonary coccidioidomycosis have markedly increased serum CRP and Hp concentrations at diagnosis that either normalize or substantially improve within 3 to 6 months after antifungal treatment. Absolute serum CRP and Hp levels are poor tests for remission in dogs with pulmonary coccidioidomycosis based on our criteria for remission. However, the percent change in serum Hp after 3 months of antifungal treatment may be useful in determining whether dogs will achieve remission within 12 months after antifungal administration.

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Disclosures

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**References**


**Supplementary Materials**

Supplementary materials are posted online at the journal website: avmajournals.avma.org.