

The utility of *Coccidioides* antigen and antibody detection in cerebrospinal fluid in the diagnosis of canine central nervous system coccidioidomycosis

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OBJECTIVE

To evaluate the utility of enzyme immunoassays (EIAs) for the detection of *Coccidioides* antigen and antibody in CSF in the diagnosis of CNS coccidioidomycosis in dogs.

ANIMALS

51 dogs evaluated for CNS disease in a single specialty center in Tucson in 2016.

PROCEDURES

Excess CSF after routine analysis was banked after collection from dogs presented to the neurology service. Samples were tested by EIA for presence of *Coccidioides* antigen and antibody. Clinical data were collected from medical records retrospectively.

RESULTS

22 dogs were diagnosed with CNS coccidioidomycosis (CCM) or another neurologic disease (non-CCM). These groups of dogs overlapped in the presenting complaints, MRI results, and routine CSF analysis results. Four dogs, all with CCM, had positive antigen EIA results. With clinical diagnosis used as the reference standard, CSF antigen testing had low sensitivity (20%) but high specificity (100%) for diagnosis of CCM. Ten dogs with CCM and 4 dogs with other diagnoses had antibody detected in CSF by EIA. Sensitivity of CSF antibody testing was 46%, specificity was 86%, and positive and negative predictive values for the study population were 71% and 68%, respectively.

CLINICAL RELEVANCE

Diagnosis of CNS coccidioidomycosis in dogs in an endemic region was hampered by overlap of clinical signs with other neurologic disorders and the low sensitivity of confirmatory diagnostics. The evaluated *Coccidioides*-specific EIAs performed on CSF can aid in the diagnosis. A prospective study is warranted to corroborate and refine these preliminary findings

Coccidioidomycosis, a systemic fungal infection caused by the dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii*, is a common condition in dogs in Arizona, southern and central California, and other parts of the southwestern US.¹ Although it most commonly presents as a fungal pneumonia, the infection can disseminate to other sites, including the CNS.²⁻⁴ Historically, the most common clinical sign related to coccidioidal brain infection in dogs is seizures,^{2,5,6} and the most common imaging, gross, and histopathological findings are 1 or more granulomatous mass lesions with coccidioidal spherules.^{3,7} However, Spoor et al⁸ reported coccidioidal encephalopathy diagnosed by MRI, positive results of anti-*Coccidioides* serologic tests, and response to antifungal treatment.⁸ Davidson et al³ also reported that MRI demonstrated meningoencephalitis in dogs with CNS coccidioidomycosis, with dogs having a wider

range of clinical signs than seizures alone. There is overlap of the known clinical and diagnostic findings in dogs with CNS coccidioidomycosis and other neurologic diseases, including neoplasia, toxins, and inflammatory diseases.

Diagnosis of dogs with CNS coccidioidomycosis is hindered by infrequent definitive identification of the pathogen in affected anatomic sites while the patient is alive due to cost, invasiveness of sample collection, and low rate of identifying organisms in CSF or other CNS tissue. Diagnostic criteria are well established for coccidioidal meningitis in humans. These criteria include symptoms of meningitis, coupled with serum antibody detection or definitive diagnosis of coccidioidomycosis in another site, and CSF abnormalities defined as elevated protein, decreased glucose, and pleocytosis.^{4,9} Cerebrospinal fluid abnormalities and presence of anti-*Coccidioides* antibody have not been comprehensively

reported for dogs with CNS coccidioidomycosis, and diagnosis is typically based on clinical signs and positive serology results or a history of coccidioidomycosis in dogs living in regions endemic for the disease.^{7,10}

Fungal culture is a low-yield (7%) diagnostic test for *Coccidioides* infection in humans,¹¹ and identification of any etiologic agent in the CSF in dogs is uncommon as well (2%).¹² Other *Coccidioides*-specific CSF testing has been studied in humans.¹³⁻¹⁵ In 1 such study,¹¹ anti-*Coccidioides* antibody was detected by enzyme immunoassay (EIA) in the CSF of 85% of patients diagnosed with *Coccidioides* meningitis. *Coccidioides* antigen detection by EIA was found to have a sensitivity of 93% and a specificity of 100% in the CSF of the same patient cohort.¹¹ To date, *Coccidioides*-specific diagnostic tests have not been evaluated for use with canine CSF. The goal of the study reported here was to determine whether detection of *Coccidioides* antigen or antibody in canine CSF by EIA corresponded with the clinical diagnosis of CNS coccidioidomycosis in dogs and offered improved diagnosis or verification of infection.

Materials and Methods

CSF samples

Cerebrospinal fluid was collected in the course of diagnostic evaluation of dogs presenting to the neurology service at the Veterinary Specialty Center of Tucson with signs of intracranial or spinal cord disease. At this hospital, excess CSF following CSF analysis was routinely archived in cryovials and stored at -20 °C, creating a bank of specimens for study. All specimens that were collected in 2016 were tested by MiraVista *Coccidioides* antigen EIA (AgEIA) and MiraVista canine *Coccidioides* IgG EIA (AbEIA). Samples were submitted in batches of approximately 15 to 20 to MiraVista Diagnostics for the panel of tests. The frozen samples were shipped overnight in 3 batches approximately 4 months apart, with the final batch shipped in early 2017. The AgEIA was performed as previously described and was considered positive at ≥ 0.07 ng/mL.¹¹ The AbEIA for canine anti-*Coccidioides* IgG was performed as previously described, and results were considered positive with a result of ≥ 10 EIA units.^{16,17} The investigators were blinded to patient data prior to CSF analysis.

Review of records

Records from dogs for which CSF was tested were reviewed retrospectively. The following information was collected: signalment, history, and clinical signs; results of routine CSF analysis, imaging, and serum *Coccidioides* agar gel immunodiffusion (AGID) assay; clinical diagnosis; treatment; and outcome, if available.

Case definition

Cases of CNS coccidioidomycosis (CCM) were identified by the attending board-certified neurologist at the time using a combination of history, clinical

signs, imaging results, CSF analysis results (cell count, cell differential, and protein concentration), and serum AGID antibody results. Histologic examination was performed on available specimens, which included postmortem brain samples that had been archived in neutral-buffered 10% formalin ($n = 4$ dogs) and a biopsy specimen from 1 dog that had undergone mass removal.

Statistical analysis

Sensitivity, specificity, and positive and negative predictive values for the AgEIA and AbEIA were performed with statistical software (Prism version 8.0; GraphPad Software Inc).¹⁸ Continuous data were assessed for normality using the D'Agostino-Pearson test,¹⁹ and the nonparametric Mann-Whitney *U* test was performed to compare age between dogs with CCM and dogs with another neurologic disease (non-CCM). The Fisher exact test was used to compare sex distributions between the CCM and non-CCM groups.²⁰ Results were considered significant at $P \leq 0.05$.

Results

CSF samples and dogs

Cerebrospinal fluid samples from 55 dogs and 2 cats were collected in 2016. Only samples from dogs were included in the study. Four (7%) of the 55 samples were removed because the dogs had incomplete medical records, leaving 51 dogs in the study. Twenty-two (43%) of these dogs were diagnosed clinically with probable or suspected CCM, while the other 29 (57%) dogs received an alternate diagnosis (non-CCM group; **Supplementary Table S1**).

Dogs ranged in age from 9 months to 13 years (mean, 6.9 years; SD, 3.1 years). All but 3 dogs (2 males and 1 female) were neutered, including 27 castrated males and 21 spayed females. There were 14 mixed-breed dogs and 27 dogs of specific breeds, with more than 1 dog classified as Golden Retriever ($n = 4$), Miniature Schnauzer (3), German Shepherd Dog (3), Greyhound (2), Russell Terrier (2), Weimaraner (2), Standard Poodle (2), and Cocker Spaniel (2). There was no difference in the median age of dogs in the CCM and non-CCM groups (7.25 and 7 years, respectively; $P = 0.29$), nor did sex distribution differ between these 2 groups ($P = 0.57$).

Clinical signs

Seizures were the most common presenting complaint, seen in over half (53% [27/51]) of the entire cohort and was the most commonly reported clinical complaint among both groups of dogs (55% [12/22] of dogs in the CCM group; 52% [15/29] of dogs in the non-CCM group). There was extensive overlap in clinical signs between these 2 groups. Dogs in both groups presented with lethargy, circling, ataxia, head tilt, behavior change, disturbances in vision or other cranial nerve deficits, tetra- or paraparesis, signs of neck or back pain, and head pressing.

History of coccidioidomycosis

Six of 22 dogs (27%) in the CCM group had historical or known concurrent coccidioidomycosis in other sites, whereas only 2 of 29 (7%) dogs in the non-CCM group had any diagnosis of coccidioidomycosis.

Clinical diagnosis

Twenty (91%) dogs in the CCM group had mass lesions or meningoencephalitis on their MRI examination, which was consistent with the literature on canine CNS coccidioidomycosis.^{3,7,8} Twenty-seven (93%) dogs in the non-CCM group had MRI performed, and in 15 (56%) a mass lesion or meningoencephalitis was identified. Routine CSF analysis was performed for all dogs in the CCM group and 27 (93%) dogs in the non-CCM group (2 dogs were euthanized after MRI). Fifteen of 21 (71%) dogs with CCM had pleocytosis (1 was excluded because of hemodilution), and 12 of 20 (60%) of these dogs (2 had missing data) had an elevated CSF protein concentration (mean, 105.6 mg/dL; range, 11.1 to 551.2 mg/dL; reference range, 14 to 30 mg/dL). Pleocytosis was noted in 7 of 26 (27%) dogs in the non-CCM group (1 was excluded because of hemodilution, and 2 were not done), while 11 of 27 (41%) had an elevated CSF protein concentration (mean, 71.6 mg/dL; range, 12 to 605.2 mg/dL).

Serum anti-*Coccidioides* antibody testing by AGID assay was performed at several different commercial laboratories, with IgG titers determined for dogs with positive results. All 22 dogs with CCM had *Coccidioides* serology performed, of which 17 (77%) were positive, with IgG titers ranging from 1:2 to $\geq 1:256$. Five of the 19 dogs in the non-CCM group that had *Coccidioides* serology performed had positive results, with titers ranging from 1:4 to 1:64. All but 1 dog with CCM were treated by oral fluconazole administration at the time of diagnosis; the remaining dog died shortly after presentation. Five dogs in the non-CCM group received oral fluconazole initially because coccidioidomycosis was considered a differential diagnosis, but the medication was discontinued when an alternate diagnosis was made.

CSF testing for *Coccidioides* antigen and antibody

There was insufficient volume of CSF from 4 dogs to perform the AgEIA for all dogs, but all samples were tested for antibody by AbEIA. With the clinical diagno-

sis used as the reference standard, sensitivity, specificity, and positive and negative predictive values were calculated for both tests (**Table 1**). Four dogs were positive by AgEIA, and all of them had a clinical diagnosis of CNS coccidioidomycosis. None of the dogs in the non-CCM group tested positive for antigen. Overall, the AgEIA had a low sensitivity for detecting *Coccidioides* infection (20%), but the specificity was 100% based on the clinical diagnosis for these cases.

A total of 14 dogs tested positive for anti-*Coccidioides* antibody (≥ 10 EIA units) in CSF; 10 were in the CCM group, and 4 were in the non-CCM group (2 had brain tumors, 1 had meningitis-vasculitis, and 1 had idiopathic epilepsy). The AbEIA had low sensitivity (46%) but high specificity (86%). This specificity was higher than that of the serum antibody AGID assay (86% vs 74%).

Discussion

Results of our study confirmed that the diagnosis of CNS coccidioidomycosis in dogs presents a challenge due to nonspecific clinical signs and routine laboratory diagnostic tests as well as difficulty obtaining invasive samples for etiologic confirmation. In addition, the presence of serum antibody against *Coccidioides* spp in healthy dogs in a highly endemic area obfuscates interpretation of serology results, with 1 study²¹ demonstrating overlap in *Coccidioides* titers up to 1:16 in dogs with clinical and subclinical infections.²¹ The AgEIA and AbEIA of CSF samples showed good specificity but relatively low sensitivity in this study. The predictive value of the AbEIA suggested that in the study cohort there was a correlation between anti-coccidioidal antibodies in the CSF and a diagnosis of CCM. However, failure to find antibodies in either serum or CSF is not sufficient to exclude CNS coccidioidomycosis as a differential diagnosis, and further diagnostic investigation is essential.

A positive AgEIA result for CSF was strongly associated with a clinical diagnosis of CNS coccidioidomycosis in this cohort of dogs. This is consistent with what is reported for humans with *Coccidioides* meningitis.²² However, in the dogs of the present study, the test was insensitive based on the clinical diagnosis on record, which is in huge contrast to the sensitivity of the test in humans with *Coccidioides* meningitis (93%).^{15,22} The AgEIA is also insensitive for

Table 1—Utility of 3 assays for the diagnosis of CNS coccidioidomycosis in dogs with CNS disease (n = 51).

Assay (sample type)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
AgEIA (CSF)	20 (6–44)	100 (87–100)	63 (57.5–67.8)	66 (51–79)
AbEIA (CSF)	46 (24–68)	86 (68–96)	71 (48–87)	68 (58–92)
AGID (serum)	77 (55–92)	73.7 (49–91)	77 (61–88)	74 (55–86)

Data are given as percentages. A clinical diagnosis of CNS coccidioidomycosis was used as the reference standard.

AbEIA = Canine *Coccidioides* IgG enzyme immunoassay. AgEIA = *Coccidioides* antigen enzyme immunoassay. AGID = Agar gel immunodiffusion assay for antibody against *Coccidioides* spp. NPV = Negative predictive value in the studied population. PPV = Positive predictive value in the studied population.

diagnosis of coccidioidomycosis generally in dogs using serum and urine.²³ However, it is a simple and specific test if CSF is being collected as part of the workup, and this EIA merits further investigation in a prospective study, especially a study that includes confirmed etiologic diagnoses.

Compared with results for the AgEIA, results for the AbEIA corresponded more frequently with the clinical diagnosis. It is notable that the AbEIA had only moderate sensitivity for the detection of *Coccidioides* antibodies in CSF. Our results indicated that while AbEIA was unable to identify all dogs with CNS coccidioidomycosis, those dogs with a positive result were likely to have neurologic coccidioidomycosis.

There was good overall agreement between the presence of anti-*Coccidioides* antibody in CSF and serum of dogs. However, due to the retrospective nature of this study, we were unable to compare CSF and serum antibodies in this cohort. Specifically, we could not determine whether there was intrathecal production of the antibodies or transfer of serum antibodies into the CSF through a leaking blood-brain barrier.^{24,25} Four dogs in the non-CCM group had anti-*Coccidioides* antibody in both serum and CSF, and it is possible that CNS inflammation in these dogs led to antibody leakage from serum to CSF.^{24,25} One of these dogs was determined to have idiopathic epilepsy based on a clinical response to antiepileptics alone over several years, and MRI showed postictal inflammation at the time the CSF sample was collected. The other 3 dogs had histopathologic findings that confirmed neoplasia (1 dog with neuroblastoma and 1 with meningioma) or severe suppurative meningitis-vasculitis and mild nonsuppurative encephalitis without evidence of an infectious agent (1 dog). The dog with meningitis-vasculitis had been diagnosed with disseminated coccidioidomycosis prior to the onset of neurologic signs and was of an age typical for idiopathic meningitis-arteritis. While several sections of the limited brain sample from this dog were histologically examined, no etiologic agent was identified. These cases underscore that the diagnosis of CNS coccidioidomycosis is more complicated in a region highly endemic for the disease.

Limitations of the present study included the retrospective design and lack of evidence available through histologic examination, cytologic examination, and microbial culture for verifying the utility of the AbEIA and AgEIA to accurately identify dogs with CNS coccidioidomycosis. Lack of proof of CNS coccidioidomycosis is typical of what practitioners face for reasons already discussed, although response to treatment of infection in the highly endemic area in which this study was performed is reasonably strong evidence. The criteria for diagnosis of *Coccidioides* meningitis in humans also do not include a requirement for identification of the organism in CSF or CNS tissues.²⁶

An advantage of the study design was that the testing of CSF was performed completely without knowledge of the dogs' diagnoses. However, this ad-

vantage was dampened by the lack of systematic collection of necessary data for included dogs, including the lack of serum anti-*Coccidioides* antibody testing, CBCs, and serum chemistry panels concurrent with the imaging and CSF analysis. *Coccidioides* AGID serology results were mined out of the patient record and were not necessarily performed at the same time as CSF collection and imaging. The AGID assay was also run at multiple commercial laboratories. A prospective study evaluating the use of CSF AgEIAs and AbEIAs with contemporaneous and uniform data would be useful in developing diagnostic criteria for CNS coccidioidomycosis. Performing more biopsies on suspected cases to prove the presence of *Coccidioides* would be ideal, but the invasiveness and cost of such procedures are likely to remain obstacles to doing so.

The spread of *Coccidioides* spp to the CNS is one of the most severe consequences of coccidioidomycosis in people and dogs. Although coccidioidomycosis is typically considered a disease that causes brain masses in dogs, the findings in this study suggested that meningoencephalitis without masses is common. Confirmation of a diagnosis by identification of organisms would be ideal but invasive. *Coccidioides*-specific CSF tests for dogs show potential as adjunctive confirmatory diagnostics, but prospective studies to further assess these assays are needed.

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Supplementary Materials

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