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STANDARD ARTICLE

# Utility of C-reactive protein and serum amyloid A in the diagnosis of equine protozoal myeloencephalitis

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**Background:** Accurate antemortem EPM diagnosis requires evidence of intrathecal antibody production. Some advocate the use of acute phase proteins in addition to serology, which alone results in substantial false positives.

**Hypothesis/Objectives:** The purpose of this study was to determine if serum C-reactive protein (CRP) or serum amyloid A (SAA) concentrations were elevated in cases of equine protozoal myeloencephalitis (EPM) compared to other neurological diseases.

**Animals:** 25 clinical cases of equine neurological disease: EPM (10), cervical vertebral stenotic myelopathy (CVSM) (10), neuroborreliosis (2), equine motor neuron disease (1), degenerative myelopathy (1), and leukoencephalomalacia (1).

**Methods:** Serum and CSF CRP and SAA were measured. Selection criteria included neurologic disease, antemortem diagnosis of EPM or CVSM, or postmortem diagnosis of EPM, CVSM, or other neurologic disease, and availability of serological results and archived samples for testing.

**Results:** Serum SAA and serum CRP levels were generally undetectable or low in horses with EPM (median CRP ≤0.1 mg/L, ≤0.1-14.4 mg/L; median SAA ≤0.1 mg/L, ≤0.1-6.11 mg/L) and CVSM (median CRP ≤0.1, ≤0.1-2.41 mg/L; median SAA ≤0.1mg/L, ≤0.1-13.88 mg/L). CSF CRP and SAA for horses with EPM (median CRP 3.35 mg/l, 0.19-13.43 mg/l; median SAA ≤0.1 mg/L, ≤0.1-2.4 mg/L) and CVSM (median CRP 4.015 mg/L, 0.16-9.62 mg/L; median SAA 0.62 mg/L, ≤0.1-2.91 mg/L) were also undetectable or low. Kruskal–Wallis test showed no statistically significant differences between serum CRP ($P$ = .14), serum SAA ($P$ = .79), spinal fluid CRP ($P$ = .65), or spinal fluid SAA between horses with EPM and CVSM ($P$ = .52).

**Conclusion:** Neither SAA nor CRP in serum or CSF aid diagnosis of EPM.

**KEYWORDS**

acute phase protein, myelopathy, *Sarcocystis neurona*, wobbler

## 1 | INTRODUCTION

Antemortem diagnosis of equine neurologic disease is challenging and definitive diagnosis often requires postmortem examination. Although equine protozoal myeloencephalitis (EPM) is the most common cause of infectious neurological disease in horses in North America, its diagnosis can be frustrating due to lack of pathognomonic signs and enigmatic immunopathogenesis. Additionally, there is widespread exposure of horses to the primary causative agent *Sarcocystis neurona*; seroprevalence in the United States ranges from 33% to 89% depending on the region.[1] However, annual incidence of clinical disease is still <1%[2] and is thought to occur when parasites invade and propagate in the CNS.

Presumptive diagnosis of EPM requires signs of neurological dysfunction consistent with EPM, ruling out other causes of disease, and immunodiagnostic testing of serum and CSF to confirm intrathecal antibody production against *S. neurona* or *N. hughesi*.[1] One test of

**Abbreviations:** App, acute phase protein; CRP, C-reactive protein; CSF, cerebrospinal fluid; SAA, serum amyloid A; Se, sensitivity; Sp, specificity; SRMA, steroid responsive meningitis-arteritis

intrathecal antibody production, the SnSAG 2, 4/3 serum: CSF titer ratio, has a sensitivity of 93.2% and specificity of 81.1% using a ratio cutoff ≤100,[2] which is recommended to avoid false negatives in clinical cases which could result in withholding treatment. In research settings, a more rigorous SnSAG 2, 4/3 serum: CSF titer ratio cutoff ≤50 is advisable to increase specificity to 95.9%.[2] Many practitioners in the field, however, rely on serum tests, which have a substantial false-positive rate due to widespread exposure to these protozoa (serum Sn SAG 2, 4/3 se = 71, sp = 50%, accuracy = 56% or IFAT se = 59%, sp = 71%, accuracy = 68%).[3]

Recently, some authors have suggested that clinical EPM might be the result of a systemic inflammatory response and have advocated the use of acute phase protein levels combined with serology to support a diagnosis.[4] Acute phase proteins (APP) are produced by the liver, and serum concentrations change rapidly in response to infection and inflammation. C-reactive protein has been extensively studied in a multitude of infectious, traumatic, and inflammatory disorders in people.[5] It is the most heavily researched APP in both human and canine neurologic disease.[5–7] Serum CRP is a minor APP in the horse with elevations detected in horses with pneumonia, enteritis, arthritis, experimentally induced laminitis, and in aseptic inflammation induced by intramuscular turpentine injection.[8] Serum Amyloid A (SAA) is the major equine acute phase protein with elevation detected in numerous conditions including arthritis, sepsis, pneumonia, abscesses, *Streptococcus equi* ssp. *equi* infections, viral infections, colic and reproductive disease.[9] However, limited information is available regarding the effect of neurologic disease on SAA levels in any species.

The purpose of this study was to investigate whether measurement of APPs was useful in differentiating infectious from noninfectious equine neurologic diseases. Specifically, the goal was to determine if CRP or SAA increase in serum or cerebrospinal fluid in horses with EPM compared to horses with cervical vertebral stenotic myelopathy (CVSM). The focus was on these diseases because they represent the most common infectious (EPM) and noninfectious (CVSM) neurologic conditions in our patient population.

## 2 | MATERIALS AND METHODS

Serum samples were selected from a repository of paired serum and CSF samples from horses with neurologic disease. These samples were collected as part of the routine diagnostic evaluation of these horses, and excess volumes were stored at −80°C until analysis. Serum and CSF CRP and SAA were measured for 25 cases of equine neurologic disease [EPM [10], CVSM [10], Lyme neuroborreliosis (LNB, 2), equine motor neuron disease (EMND, 1), equine degenerative myeloencephalopathy (EDM, 1), and leukoencephalomalacia [1]] diagnosed as follows. Nine of 10 EPM cases had serum: CSF SnSAG2, 4/3 titer ratios ≤25, and one was not tested. The untested EPM case was confirmed postmortem via immunohistochemistry for *S. neurona*. Four of the tested EPM cases also had postmortem histologic diagnoses of EPM. Nine of the 10 CVSM cases were confirmed postmortem, and the other CVSM case had spinal cord compression confirmed by myelogram and SnSAG2, 4/3 titer ratio of 200. The remaining cases were diagnosed on postmortem examination.

Serum and cerebrospinal fluid CRP levels were measured using an automated analyzer (RX Daytona Analyzer, Randox, Kearneysville, VA) and the antihuman CRP reagent (Randox high linearity CRP reagent, Randox, Kearneysville, VA) as previously described for use with canine samples.[10] CRP levels in normal animals are considered negligible (≤0.1 mg/L); however, previously reported plasma concentrations for reported inflammatory conditions range from 10 to 35 mg/L.[8] The same method for serum testing was also applied to cerebrospinal fluid CRP measurement. There currently are no established reference ranges for serum or cerebrospinal fluid CRP values in horses using this method.

SAA concentration was quantitated with a kit (Eiken, Tokyo, Japan) on an automated analyzer (Daytona Analyzer, Randox, Kearneysville, VA) as previously described.[11,12] The analyzer was subject to routine quality control measurements throughout the study. This assay has previously been described as having acceptable linearity within clinically relevant ranges of SAA concentration in horses.[11,12] On the basis of in-laboratory–derived reference intervals, which were consistent with previously published data[11,12] serum SAA concentrations ≥20 mg/L were considered abnormally high. The same turbidometric immunoassay was also used for SAA measurement in cerebrospinal fluid; however, no cerebrospinal fluid SAA reference ranges have been established using this method.

A Kruskal–Wallis equality-of-populations rank test was applied to determine if there is a 1) difference in C-reactive protein (CRP) or serum amyloid A (SAA) measured in either serum or spinal fluid in cases of equine protozoal myeloencephalitis (EPM) and cervical vertebral stenotic myelopathy (CVSM) and 2) if there is a difference in CRP or SAA measured in either serum or spinal fluid in cases of infectious neurological disease (EPM + neuroborreliosis) and noninfectious neurological disease (CVSM + EMND+EDM+ leukoencephalomalacia). Statistical analysis was performed using computer software (STATA 14 MP, StataCorp, College Station, TX).

## 3 | RESULTS

Only one case of EPM had a mild elevation in serum CRP of 14.4 mg/L, but no other elevations in serum SAA or serum CRP were detected in cases of EPM (median CRP ≤0.1 mg/L, range ≤0.1-14.4 mg/L; median SAA ≤0.1 mg/L, range ≤0.1-6.11 mg/L) or in any cases of CVSM (median CRP ≤0.1, range ≤0.1-2.41 mg/L; median SAA ≤0.1mg/L, range 0.1-13.88 mg/L). In cases of EPM serum SAA and CRP levels exceeded the minimum detectable value of 0.1 mg/L in 4 cases for each variable and only one horse had measurable levels of both acute phase proteins though neither exceeded reference values. In cases of CVSM, serum CRP exceeded the minimum detectable value of 0.1 mg/L in only 1 case, and the SAA value exceeded the minimum detectable value in only 3 cases with no values exceeding reference ranges. The case of leukoencephalomalacia had a mild increase in serum CRP (12.71 mg/L) with no detectable serum SAA (≤0.1 mg/L). The two cases of Lyme neuroborreliosis had no increase in serum CRP (≤0.1mg/l); however, mild to moderate elevation in serum

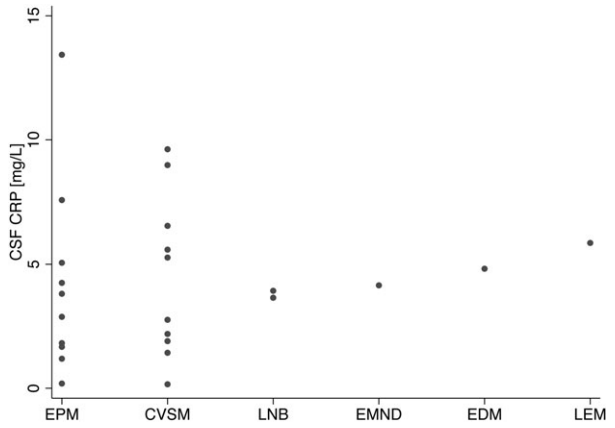


**FIGURE 1** Comparison of Cerebrospinal Fluid (CSF) C-Reactive protein (CRP; mg/L) in cases of Equine Protozoal Myeloencephalitis (EPM; n=10), Cervical Vertebral Stenotic Myelopathy (CVSM; n=10), Lyme Neuroborreliosis (LNB; n=2), Equine Motor Neuron Disease (EMND; n=1), Equine Degenerative Myelopathy (EDM; n=1), and leukoencephalomalacia (LEM; n=1)

SAA was detected (132.06 mg/L and 459.81 mg/L; ref range 0-20 mg/L). No consistent relationships between SnSAG 2, 4/3 antibody levels, and serum CRP or SAA were detected nor was there a relationship between the two acute phase proteins in cases of EPM (Supporting Information). Serum CRP and SAA for horses with the other conditions were as follows: EMND ≤0.1 mg/L, 4.77 mg/L; EDM 4.23 mg/L, ≤0.1 mg/L; leukoencephalomalacia 12.71 mg/L, ≤0.1 mg/L.

Similar to serum results, there was considerable overlap in CSF values of CRP and SAA across all neurological diseases (Figures 1 and 2). Cerebrospinal fluid CRP and SAA values in cases of EPM (CRP median 3.35 mg/L, range 0.19-3.43 mg/L; SAA median ≤0.1 mg/L, range ≤0.1-2.4 mg/L) did not differ significantly from those with CVSM (CRP median 4.015 mg/L, range 0.16-9.62 mg/L; SAA median 0.62 mg/L, range ≤0.1-2.91 mg/L). CSF CRP and SAA for the other conditions are as follows: EMND 4.15 mg/L, ≤ 0.1 mg/L; EDM 4.82 mg/L, ≤0.1 mg/L; leukoencephalomalacia 5.86 mg/L, 0.23 mg/L. We did not observe statistically significant differences in serum CRP ($P$ = .14), serum SAA ($P$ = .79), spinal fluid CRP ($P$ = .65), or spinal fluid SAA ($P$ = .52) between horses with EPM and CVSM. Furthermore, no statistical differences were observed between infectious (EPM + neuroborreliosis) and noninfectious diseases (CVSM + EMND + EDM+ leukoencephalomalacia) for serum CRP ($P$ = .63), serum SAA ($P$ = .79), CSF CRP ($P$ = .41), or CSF SAA ($P$ = .81).

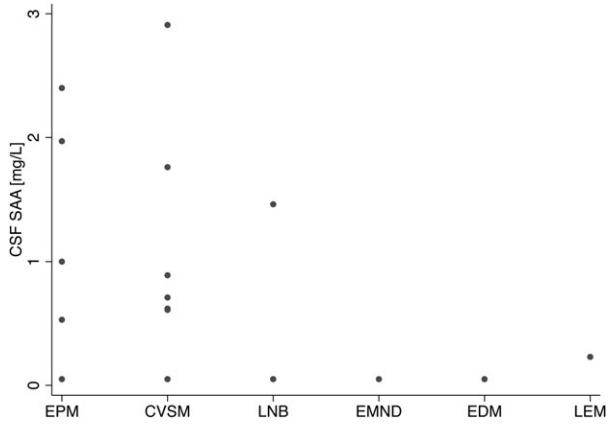


**FIGURE 2** Comparison of Cerebrospinal Spinal Fluid (CSF) Serum Amyloid A (SAA; mg/L) in cases of Equine Protozoal Myeloencephalitis (EPM; n=10), Cervical Vertebral Stenotic Myelopathy (CVSM; n=10), Lyme Neuroborreliosis (LNB; n=2), Equine Motor Neuron Disease (EMND; n=1), Equine Degenerative Myelopathy (EDM; n=1), and leukoencephalomalacia (LEM; n=1)

## 4 | DISCUSSION

Based on this study, measuring serum CRP and SAA does not aid in making the diagnosis of equine neurologic disease. Results from this study do not support the use of either serum CRP or SAA in differentiating horses with EPM from those with CVSM. Failure to detect increases in APPs in horses with EPM could indicate that *Sarcocystis neurona* infection does not incite a systemic inflammatory response. Alternatively, this finding could indicate that horses do not develop clinical signs of neurologic disease until the systemic inflammatory response has waned.

Serum CRP concentration is not elevated in dogs with necrotizing meningoencephalitis nor in several types of brain tumors leading to the conclusion that one cannot determine intracranial inflammation based on serum CRP concentration alone.[13] Additionally, CRP concentration was not elevated in cases of intervertebral disk protrusion or degenerative lumbosacral stenosis.[13] The lack of elevation in CRP and SAA in cases of EPM and CVSM mirror these results in dogs.

Serum SAA was elevated in both cases of Lyme neuroborreliosis. Because only two horses with this condition were included in this study further research is warranted. Both horses displayed dysphagia and were diagnosed with secondary aspiration pneumonia, which might account for the increases in SAA. Alternatively, the rise in SAA might be due to systemic effects of *Borrelia burgdorferi*, which can result in multisystemic inflammatory disease including meningitis, polyradiculoneuritis, myositis, myocarditis, vasculitis, uveitis, and polyarthritis.[14,15] Both horses had evidence of inflammatory disease within and outside of the nervous system; one had myositis and laminitis while the other horse with the greater elevation in SAA had severe uveitis. Despite having elevated SAA levels, neither horse with neuroborreliosis had increased serum CRP concentration, which is consistent with a large human study of neuroborreliosis, in which CRP was only elevated in 15% of cases.[16]

Although horses in this study did not demonstrate even modest increases in CSF CRP levels in most cases, there might be practical utility in measuring CRP in the CSF of horses with other types of neurologic conditions, such as neurotrauma or equine herpesvirus myeloencephalopathy. An increase in CRP concentration in CSF is thought to largely result from blood-brain barrier damage (ex. subarachnoid hemorrhage[6] or vasculitis due to sepsis[17]); intrathecal production of CRP[18] and disruption of glymphatic circulation may also contribute to a lesser extent.[19] Even when the blood–brain barrier is intact CRP level in the CSF is directly related to serum CRP because this protein flows from blood into the cerebrospinal space,[20] but determination of a CRP index along with IgG index has been proposed in cases of

canine Steroid Responsive Meningitis-Arteritis (SRMA)[6] and may also be helpful in cases of blood–brain barrier disruption in equine neurological disease.

Potential confounding factors exist that could interfere with accurate measurement and interpretation of serum SAA and CRP. Any inflammatory process within the horse could cause a rise in acute phase proteins. Many horses are exposed to *Borrelia burgdorferi* and subsequently develop antibodies without clinical disease; although subclinical inflammation might be present.[21,22] Horses are commonly exposed to viruses and bacteria that can lead to respiratory or gastrointestinal infections, and these problems might trigger systemic inflammation. For an individual horse with neurologic disease and increased APP levels, the underlying cause of the APP rise might not be determined definitively and caution is advised in overinterpretation of APP levels when concurrent systemic illness is suspected. Determination of specific biomarkers of nervous system disease in the horse are warranted and might be extrapolated from canine and human studies. *S100B*, colloquially referred to as the CRP of the brain, is one such protein that may be worth further investigation.[23] This protein is specific to central nervous system disease, but its diagnostic sensitivity in the horse is unknown at this time.

Limitations to this study also include small sample size of only twenty-five cases including only two cases of neuroborreliosis. In the 10 EPM cases only five had definitive diagnosis via postmortem examination. However, the tested EPM cases in this study did have serum: CSF snSAG2, 4/3 titer ratios ≤25, which corresponds to 98.8% specificity and dramatically reduces the chance of misdiagnosis.[2] All samples of CSF and serum tested were stored at −80°C prior to shipping and analysis making sample degradation an unlikely source of error. No studies have evaluated the stability of CRP in frozen equine serum and spinal fluid, but human serum has been stored up to 11 years at −80°C without any significant change.[24] Canine CRP studies have also routinely stored serum and CSF frozen (−20°C) prior to assay.[6,7,20]

In summary, neither serum C-reactive protein (CRP) nor serum amyloid A (SAA) increased significantly in cases of equine protozoal myeloencephalitis (EPM) or cervical vertebral stenotic myelopathy (CVSM), and thus do not aid in the diagnosis of either. Additional research is needed to determine if equine neuroborreliosis results in increase in SAA or if concomitant illness or systemic effects of Lyme disease are responsible for the SAA elevations detected in this study. Future research is warranted to determine if APP other than CRP or SAA measured in the serum and CSF will aid diagnosis of EPM, CVSM, or other equine neurological diseases.

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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.

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## SUPPORTING INFORMATION

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

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