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Short Communication

Comparison of Serum Amyloid A in Horses With Infectious and Noninfectious Respiratory Diseases

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ABSTRACT

The acute phase protein serum amyloid A (SAA) has been shown to be a useful inflammatory parameter in the horse, but studies showing SAA responses to specific respiratory disease etiologies are limited. The goal of this study was to evaluate SAA responses in horses with infectious and noninfectious respiratory diseases as well as healthy, control horses. Two hundred seven horses were grouped into the following categories: equine influenza virus (EIV), equine herpesvirus-4 (EHV-4), *Streptococcus equi* subspecies *equi* (*S. equi* ss *equi*), inflammatory airway disease (IAD), and healthy controls. Serum amyloid A concentrations were determined for all horses on serum using a stall-side lateral flow immunoassay test. Serum amyloid A levels were found to be significantly greater for infectious respiratory diseases (EIV, EHV-4, *S. equi* ss *equi*) and horses with IAD when compared to control horses. There was a significant difference between viral and bacterial infections and IAD. Although SAA values from horses with *S. equi* ss *equi* were significantly greater when compared to horses with viral infections (EIV/EHV-4), the wide range of SAA values precluded accurate classification of the infectious cases. In conclusion, SAA is more reliably elevated with infections of the respiratory tract rather than noninfectious airway conditions. This can facilitate early detection of respiratory infections, help track disease progression, and aid practitioners in making recommendations about proper biosecurity and isolation of potentially contagious horses.

1. Introduction

Serum amyloid A (SAA) is a major acute phase protein (APP) identified in horses that maintains very low levels in health and increases up to 5,000 to 10,000 mg/L in animals with severe inflammation [1,2]. It has become a useful maker for inflammation, as it rapidly increases in concentration over 6 to 12 hours, much faster than the greater than 72 hours response time identified for fibrinogen, a minor APP [3]. In addition, SAA has a short half-life, which results in a more rapid decrease in concentration and an ability to assess response to treatment and changes in disease process [3]. Serum amyloid A was studied as a marker for inflammation in horses affected with equine influenza and found to peak during the acute stages of infection and did not return to baseline until 11 to 22 days later, for uncomplicated cases [4]. Conversely, SAA was studied in racehorses with inflammatory airway disease (IAD) and was not increased when compared to healthy controls [5]. Infectious and noninfectious conditions of the upper respiratory tract of horses can present similarly, with poor performance, exercise intolerance, coughing, and nasal discharge. The presence of fever and multiple horses affected on a premise are criteria often used to gage the infectious and contagious nature of an upper airway disease. It is therefore imperative that practitioners use clinical and laboratory information to minimize spread of

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infectious respiratory viruses and bacteria. The purpose of this study was to evaluate the response of SAA with inflammatory infectious (equine influenza virus [EIV], equine herpesvirus-4 [EHV-4], and *Streptococcus equi* subspecies *equi* [*S. equi* ss *equi*]) and inflammatory/noninfectious (IAD) respiratory diseases using a stall-side lateral flow immunoassay test.

2. Materials and Methods

For this study, convenience serum samples collected from 207 horses of varying ages, breeds, and genders were used (Table 1). The study horses were selected from various repository sample collections and assigned to an infectious respiratory disease group, an IAD group, or a control group based on the following inclusion criteria. For this study, only horses with infectious respiratory disease were included if they had a fever of ≥38.5°C and respiratory signs (cough and/or nasal discharge) and a positive qPCR result of nasal secretions for EIV (n = 42), EHV-4 (n = 43), or *S. equi* ss *equi* (n = 44). Horses were assigned to the IAD group (n = 38) based on clinical signs (chronic, intermittent cough, increased mucoid airway secretions, and decreased performance), imaging findings (bronchial pattern), pulmonary function testing, and cytological analysis of broncho-alveolar lavage fluid (increased total nucleated cell count with increased neutrophils, lymphocytes, monocytes, eosinophils, and/or mast cells) in line with the ACVIM consensus statement by Coutëil et al [6]. Control horses (40) were considered healthy with a normal physical evaluation and qPCR negative results for common respiratory pathogens (EIV, EHV-1, EHV-4, and *S. equi* ss *equi*). Serum samples were all collected within 24 months of analysis and kept frozen at −80°C prior thawing.

Serum samples from all horses were tested for SAA levels using a commercially available stall-side lateral flow immunoassay test (StableLab Equine Blood Analysis Kit, Sligo, Ireland). The stall-side test has been validated by the manufacturer, and the precision and accuracy were determined to be 98.6% and 95.6% at concentrations ranging between 50 and 2,000 mg/L. In a recent preliminary validation of the analytical accuracy of the stall-side lateral flow immunoassay test, this assay showed good linearity between 0 and 2,000 mg/L with good agreement with a turbidimetric immunoassay (Eiken, Tokyo, Japan). The intraassay coefficient of variability ranged between 13% and 18% for low, intermediate, and high SAA concentrations. Furthermore, SAA concentrations in whole blood and serum/plasma were positively correlated (Diana Schwartz, personal communication). The detection range for the test is 0 to 3,000 mg/L. Samples with SAA concentrations >3,000 mg/L were not diluted to obtain an absolute concentration and were reported as 3,000 mg/L.

Differences in SAA values among the groups were determined using the exact Kruskal–Wallis test. $P < .05$ was considered statistically significant for all analyses.

3. Results

The majority of the healthy horses had undetectable SAA (minimum, 0 μg/mL; maximum, 2 mg/L; median, 0 mg/L; Fig. 1). Six horses with IAD had detectable SAA up to 586 mg/L (minimum, 0 mg/L; maximum, 586 mg/L; median 0 mg/L). The SAA for EIV qPCR positive horses ranged from

Table 1
Signalment and clinical signs of healthy horses and horses with infectious and noninfectious respiratory diseases.

Variables	EHV-4	EIV	*Streptococcus equi ss equi*	IAD	Control
Sample size	43 horses	42 horses	44 horses	38 horses	40 horses
Age (y)	Range = 0.3–13 Median = 2 Unknown = 3	Range = 0.1–20 Median = 4 Unknown = 4	Range = 0.5–29 Median = 8 Unknown = 1	Range = 3–25 Median = 10 Unknown = 0	Range = 5–21 Median = 11.5 Unknown = 0
Sex	Female = 18 Male = 24 Unknown = 1	Female = 21 Male = 18 Unknown = 3	Female = 12 Male = 29 Unknown = 3	Female = 9 Male = 29 Unknown = 0	Female = 37 Male = 3 Unknown = 0
Breed	Quarter Horse = 16 Thoroughbred = 7 Arabian = 6 Paint Horse = 5 Friesian = 2 Other = 4 Unknown = 3	Quarter Horse = 16 Welsh Pony = 3 Saddlebred = 3 Percheron = 3 Warmblood = 2 Thoroughbred = 2 Friesian = 2 Other = 8 Unknown = 3	Quarter Horse = 27 Thoroughbred = 3 Saddlebred = 3 Arabian = 3 Haflinger = 2 Other = 6 Unknown = 0	Warmblood = 11 Quarter Horse = 7 Welsh Pony = 4 Friesian = 2 Paint Horse = 2 Thoroughbred = 2 Gypsy Vanner = 2 Other = 8 Unknown = 0	Quarter Horse = 22 Thoroughbred = 10 Warmblood = 8 Unknown = 0
Rectal temperature (°C)	Range = 38.5–41.7 Median = 39.7	Range = 38.5–40.6 Median = 39.6	Range = 38.5–41.1 Median = 39.6	All afebrile (<38.5)	All afebrile (<38.5)
Cough	43/43 had cough ranging from occasional to frequent	42/42 had cough ranging from occasional to frequent	44/44 had cough ranging from occasional to frequent	30/38 had cough	0/40 had cough
Nasal discharge	43/43 had nasal discharge ranging from mild to severe	42/42 had nasal discharge ranging from mild to severe	44/44 had nasal discharge ranging from mild to severe	21/38 had nasal discharge	0/40 had nasal discharge

Abbreviations: EHV-4, equine herpesvirus-4; EIV, equine influenza virus; IAD, inflammatory airway disease.

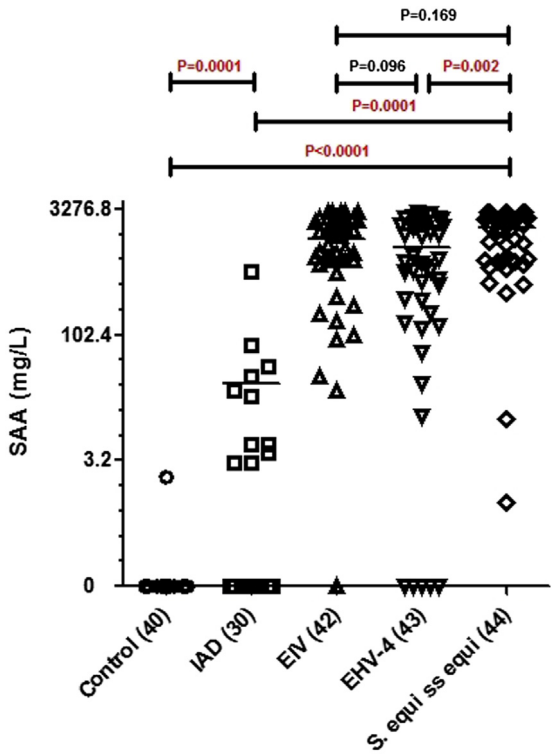

Fig. 1. Distribution of SAA values in healthy control horses; horses with IAD; and horses with EIV, EHV-4, and *Streptococcus equi* ss *equi* infection. The horizontal lines represent median SAA values. EHV-4, equine herpesvirus-4; EIV, equine influenza virus; IAD, inflammatory airway disease; SAA, serum amyloid A.

0 to ≥ 3,000 mg/L (median, 731 mg/L; five samples with SAA ≥ 3,000 mg/L). The SAA values for EHV-4 qPCR positive horses ranged from 0 to ≥ 3,000 mg/L (median, 1,173 mg/L; one sample with SAA ≥ 3,000 mg/L). The SAA for *S. equi* ss *equi* qPCR positive horses ranged from 0 to ≥ 3,000 mg/L (median, 1,953 mg/L; five sample with SAA ≥ 3,000 mg/L). There were significant differences between the control group and the various inflammatory disease groups ($P = .0001$). There was also a significant difference between horses from the IAD group and the three infectious groups ($P = .0001$). There was a significant difference between the EHV-4 group and the *S. equi* ss *equi* group ($P = .002$), but not between the EHV-4 group and the EIV group ($P = .096$) and not between the EIV group and the *S. equi* ss *equi* group ($P = .169$). When both viral disease groups were combined and compared to the *S. equi* ss *equi* group, there was a significant difference ($P = .0095$).

4. Discussion

Past studies have looked at the SAA levels in horses with EIV infection and IAD, but have not compared noninfectious with infectious respiratory diseases [4,5]. Collectively, these studies showed that SAA increases with acute inflammation from an infectious respiratory disease. A commercially available stall-side SAA lateral flow immunoassay test was recently introduced into the equine market and gives practitioners the ability to rapidly measure an important marker of inflammation.

To the authors knowledge, this is the first report comparing SAA levels in natural infections with viral and bacterial infections of the upper respiratory tract and inflammatory, noninfectious etiologies. This study confirms the diagnostic hypothesis that SAA will more reliably be elevated with infections of the respiratory tract rather than noninfectious airway conditions. The population used in this study represents horses of various ages and breeds with acute onset of respiratory signs. Furthermore, all horses with infectious and noninfectious respiratory diseases presented with coughing and nasal discharge of varying degrees. Early in the course of the disease process, equine practitioners may be faced with the challenge of differentiating disease etiologies with similar clinical pictures. Equine practitioners can use SAA during the acute onset of disease to help differentiate respiratory infections from inflammatory, noninfectious respiratory diseases showing similar clinical signs. Also, it has been shown that SAA levels remain undetectable in horses that, despite being in contact with transmissible pathogens, did not contract the disease [7]. Measuring SAA values in horses can be especially useful to track disease progression and make recommendations about biosecurity and isolation. Although there is a significant difference in SAA values between bacterial and viral infectious respiratory diseases, there is a lot of overlap in SAA values between these two groups. Therefore, it is not recommended that SAA levels alone be used to distinguish between bacterial and viral respiratory diseases. Future studies may look to grade horses with varying clinical disease and give scores based on severity of clinical signs and compare severity of inflammatory response and SAA levels to infectious etiologies.

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