Equine Veterinary Education

EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2022) **34** (12) e533-e539
doi: 10.1111/eve.13557

Original Article

Comparison of the diagnostic predictability of serum amyloid A, white blood cell count and immunoglobulin G tests as indicators of early-onset, acute-phase morbidities in newborn foals

***N. M. Nieman*[†,*] *and D. S. Chan*[‡]**

[†]*Winstar Farm, LLC Versailles, Kentucky, USA; and* [‡]*Epona Biotech Sligo, Institute of Technology Campus, Sligo, Ireland*
**Corresponding author email: nniemandvm@winstarfarm.com*

Keywords: horse; serum amyloid A; foal; diagnostic blood test; white blood cell count; immunoglobulin G

Summary

Background: Acute-phase proteins (APPs) have diagnostic value as nonspecific, early indicators of inflammation and infectious disease.

Objectives: To assess the ability of serum amyloid A (SAA) testing to distinguish between healthy and nonhealthy newborn foals, and to compare the diagnostic predictability of SAA with white blood cell (WBC) counts and serum immunoglobulin G (IgG) concentration.

Study design: Three-year prospective clinical evaluation and diagnostic sample collection from newborn foals.

Methods: Following an examination, a blood sample was obtained from each foal and tested for SAA and IgG concentrations, complete blood count (CBC) and standard biochemistry values. Foals were categorised as healthy or nonhealthy based on the clinical examination. The presence of clinical signs of infectious origin or certain noninfectious morbidities with a pro-inflammatory component was sufficient for a nonhealthy designation. Serum amyloid A, IgG and WBC test results were determined and compared for healthy and nonhealthy foals.

Results: A total of 24 (13.7%) foals were diagnosed as clinically sick and 151 (86.3%) as normal based on physical examinations performed within 24 h after parturition. Mean SAA values were 27.7 ± 129.0 mg/L and 247.2 ± 454.8 mg/L for healthy and nonhealthy foals, respectively, a significant ($p < 0.05$) difference indicating that elevated SAA values are associated with a nonhealthy diagnosis. Using a positive threshold of 100 mg/L, the SAA test had a positive predictive value (PPV) of 55.6% and IgG had the lowest PPV (16.7%) followed by WBC count (23.3%).

Main limitations: A larger number of clinically nonhealthy foals with SAA levels >100 would have given the study greater statistical power.

Conclusions: Equine practitioners can consider SAA testing to be a reliable newborn examination diagnostic tool for detecting early-onset, acute-phase infection or noninfectious morbidities with an inflammatory component.

Clinical relevance

- Serum amyloid A screening functioned as a sensitive interpretive marker of infectious and other inflammatory morbidities in neonatal foals, a high-risk equine population that potentially benefits from early therapeutic intervention.
- A novel SAA testing device validated for use in horses provided rapid and convenient point-of-care screening for SAA in an equine neonatal examination setting.

Introduction

Although pathogen-specific diagnostic testing is the gold standard for targeted therapy, this approach is of limited benefit for detecting subclinical or early-onset infection and can be time-consuming and expensive. Acute-phase proteins (APPs) have diagnostic value as nonspecific, early indicators of inflammation and infectious disease. Serologic testing for APPs can be a useful option for initiating timely therapeutic intervention pending the results of more definitive diagnostic testing. Early recognition and treatment can improve morbidity and mortality outcomes, minimise therapeutic costs and avoid long-term effects of disease.

Measuring APP status can be particularly helpful in the case of neonatal foals, which are at increased risk of infectious disease in endemic settings or if adequate transfer of maternal antibodies has not occurred within 24 h of birth. Nonspecific, pro-inflammatory serologic biomarkers commonly used for diagnostic purposes in equine medicine include white blood cell (WBC) count and fibrinogen, an APP. Compared to these traditional indicators, serum amyloid A (SAA) is an APP that is increasingly considered a more sensitive and reliable diagnostic tool in evidence-based equine practice (Jacobsen & Andersen, 2007; Nunakawa et al., 1993).

Serum amyloid A is classified as a major APP, meaning that it has an undetectable or very low basal level in the plasma of healthy individuals but increases markedly across a broad dynamic range in response to inflammatory stimuli. Typically, SAA is the initial nonspecific systemic response to pro-inflammatory infectious disruptions to homeostasis. Serum amyloid A concentrations increase >10-fold and as much as 100 times or more during acute-phase episodes (Jacobsen & Andersen, 2007; Nunokawa et al., 1993). Expression of SAA increases rapidly to high concentration in response to infection or inflammation, generally in linear proportion to the extent of tissue damage. Concentrations of SAA remain elevated as long as the infectious condition persists. Due to a very short half-life, SAA concentrations decline rapidly with disease resolution. This kinetic profile makes SAA an objective, highly sensitive, interpretive marker of inflammation, particularly of infectious origin, as well as of treatment response and recovery in horses regardless of age or gender (Jacobsen & Andersen, 2007; Nunokawa et al., 1993; Pepys et al., 1989). While serving most often as a marker of infectious disease, elevated SAA has also been associated with certain noninfectious morbidities, although not to the degree commonly found in cases of systemic infections. Significant elevations in SAA have been found in such noninfectious conditions as neonatal maladjustment syndrome (NMS), perinatal trauma, meconium colic, tissue injury, neonatal foal weakness, gastrointestinal disease and local infections (Chavatte et al., 1992; Jacobsen & Anderson, 2007; Stoneham et al., 2001).

The recent development of a novel SAA testing device validated for use in horses has made rapid and convenient point-of-care screening for SAA possible in equine practice. The commercially available test kit uses a lateral flow immunoassay of an equine serum sample to measure SAA concentration between 0 and 3000 mg/L. A handheld reader gives a quantitative SAA result within 10 min after insertion of a test sample cartridge. The precision and accuracy of the immunoassay were determined to be 98.6 and 95.6%, respectively, at concentrations between 50 and 2000 mg/L (Viner et al., 2017). Other reports have described how the test kit was used for SAA screening in clinical or investigational settings (Giguere et al., 2016; Ludwig et al., 2016; McCracken, 2019; Viner et al., 2017).

The objective of this study was to assess the reliability of SAA testing in distinguishing between healthy and nonhealthy newborn foals and to compare the diagnostic predictability of SAA with WBC counts and serum immunoglobulin G (IgG) concentration. Several determinants of diagnostic value were compared, including test sensitivity, specificity, positive and negative predictive values, and test accuracy. In contrast to a hospital or ICU setting where patients have overt clinical signs, new foal examinations tend to encounter ambiguous presentations where SAA screening can be a sensitive indicator for detecting preclinical infections. An algorithm for incorporating SAA into a neonatal foal examination protocol is described in this report.

Materials and methods

Study sites and animals

The study was conducted on a Kentucky Thoroughbred breeding farm during three consecutive breeding seasons from 2013 to 2015. The study population consisted of 175 neonatal foals that were clinically evaluated by the same resident veterinarian within 19 h post-parturition.

Study design

Following a clinical examination, a blood sample was obtained from each foal enrolled in the study and tested for SAA and IgG concentrations, complete blood count (CBC) and standard biochemistry values. Foals were categorised as healthy or nonhealthy based on the results of the clinical examination. The presence of overt clinical signs of presumptive infectious origin or noninfectious morbidities with a pro-inflammatory component such as NMS was sufficient for a nonhealthy designation. Agreement between the clinical health status of each foal and its SAA, IgG and WBC test results was determined. Normal diagnostic values were <100 mg/L for SAA, ≥88.0 g/L for serum IgG and 5–12 x 10^9/L for WBC count. Sensitivity and specificity rates were then calculated for each of the three diagnostic tests based on their overall agreement with the clinical health status of the study population.

Diagnostic testing

Serum amyloid A was measured using a commercial test cartridge and a handheld reader (SAA Equine Blood Analysis Test and EQ-1 Reader: Epona Biotech) designed for point-of-care testing. Immunoglobulin G values were determined from whole-blood samples using a semi-quantitative ELISA test (SNAP Foal IgG: Idexx). White blood cell counts were obtained using a calibrated analyser (Hemavet 850: Drew Scientific). All diagnostic samples were obtained within 19 h after birth.

Data collection and analysis

The relationship between diagnostic test results and the clinical health status of each foal was determined. Aggregate results were then analysed for specificity, sensitivity, positive predictive value and negative predictive value (MedCalc Statistical).

In a previous study (Belgrave et al., 2013), investigators evaluated the diagnostic test accuracy of SAA, plasma fibrinogen and WBC tests in clinically normal and clinically sick horses, using the formula:

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \times 100.$$

where TP = true positives, TN = true negatives, FP = false positives and FN = false negatives. Accuracy is defined as the percentage of subjects from the total population correctly diagnosed as true positives or true negatives. The application of this formula as an epidemiologic tool has been previously described (Baratloo et al., 2015). The formula was used to calculate the accuracy of the three test methods used in this study as well as the following formulas for calculations used.

Sensitivity = TP/(TP + FN) × 100 (% True positives out of Sick group).

Specificity = TN/(TN + FP) × 100 (% True negatives out of Healthy group).

PPV = TP/(TP + FP) × 100 (% True positives out of all positive results).

NPV = TN/(TN + FN) × 100 (% True negatives out of all negative results).

Chi-square and P-values were calculated using the Kruskal–Wallis test to assess the relationship between SAA values and health status. Significance was designated as $p < 0.05$.

Results

Relationship between clinical health status and SAA test results

A total of 24 (13.7%) foals were diagnosed as clinically sick and 151 (86.3%) as clinically normal based on physical examinations performed within 36 h after parturition. The majority of nonhealthy foals had enteritis, diagnostically confirmed in some foals as rotavirus diarrhoea. **Table 1** summarises the clinical signs occurring in clinically sick foals, some of which had infectious and noninfectious comorbidities.

The mean SAA value for healthy foals was 27.7 ± 129 mg/L (median 0; range, 0 to 1497). The mean SAA value for nonhealthy foals was 247.2 ± 454.8 mg/L (median, 58; range, 0–1737). The Kruskal–Wallis test found that elevated SAA values were significantly associated with a nonhealthy diagnosis ($p < 0.05$). This relationship is illustrated in **Fig 1**, showing the distribution of SAA concentration for healthy and nonhealthy foals. Three healthy foals had SAA values >200 mg/L, indicating that asymptomatic outliers above the positivity threshold occasionally occur. Conversely, 14 of 24 nonhealthy foals had a SAA value below the positivity threshold, indicating that sick animals can have a SAA level <100 mg/L.

Diagnostic test predictability

The sensitivity and specificity of the three diagnostic tests for identifying healthy and nonhealthy foals are summarised in **Table 2**. Using a threshold of 100 mg/L for indicating nonhealthy status, the SAA test had a sensitivity of 41.7% for positive diagnoses and a specificity of 94.7% for negative diagnoses. Immunoglobulin G results had a low, 8.3% sensitivity for identifying sick foals and a 93.4% specificity as an indicator of healthy status. White blood cell count had a 41.7% sensitivity as an indicator of nonhealthy status and a 78.1% specificity for identifying healthy foals.

The probability of the three diagnostic tests correctly predicting positive and negative health status in the study population is summarised in **Table 3**. The SAA test had the highest PPV (55.6%) and NPV (91.1%) rates of the three diagnostic tests. Immunoglobulin G had the lowest PPV (16.7%) followed by WBC count (23.5%). In relative terms, the SAA test had a PPV that was threefold greater than that for IgG and 2.3-fold greater than that for WBC count. All three tests had NPVs >85%, indicating that they were reasonably good surrogates for clinically healthy status. When SAA test results were considered with WBC results, the additive effect resulted in PPVs of 66.7% (**Table 3**).

Sensitivity and specificity outcomes translated into test accuracy of 87.4, 81.7 and 73.1% for SAA, IgG and WBC assays, respectively.

TABLE 1: Incidence of clinical signs in 24 foals diagnosed as nonhealthy

Clinical signs	No. foals affected*
Enteritis	14
Neonatal maladjustment syndrome	10
Ileus	1
Umbilical sepsis	1
Tachypnoea	2
Birth trauma, musculoskeletal fractures, congenital abnormalities	4
Weak, unthrifty	2

*Some foals had >1 clinical sign or syndrome or had concurrent laboratory test values outside normal ranges.

Discussion

By any of the diagnostic measures, SAA at a positivity threshold of 100 mg/L was superior to IgG levels and WBC count as an indicator of health status of the study population. The difference was particularly pronounced when SAA was compared to WBC count, a routinely used nonspecific indicator of host immune-response status. In relative terms, SAA testing had a more than twofold greater PPV, 10.0% greater sensitivity, 18.0% greater specificity and 15.0% greater test accuracy compared to WBC count. When WBC count is used as a diagnostic indicator in foals, concentrations ≥13.0 x 10^9/L have been shown to have a sensitivity and specificity for *R. equi* of 95.2% and 61.2%, respectively (Giguere et al., 2003), indicating that it remains a valid diagnostic biomarker. In neonates, serum IgG concentration below the normal range indicate suboptimal transfer of colostrum antibodies. Serum IgG was a poor predictor of nonhealthy status (16.7% PPV) and in a naïve, neonatal population would have greater value as an indicator of immune status and infection risk. In our study, the 19-h postpartum interval during which the neonatal examination was performed was probably too short for IgG to be a reliable indicator of health status in all cases. In neonates, the value of SAA as a diagnostic indicator is generally greatest when it is based on samples obtained as soon as possible after parturition and is followed by repeat testing when indicated. Because of the short half-life of SAA in vivo, the longer sample collection is delayed, the less likely it is that the SAA level will reflect an acute-phase response.

Eight clinically normal foals had SAA values >100 mg/L. Three of these foals had SAA concentration more than double the positivity threshold (272, 391 and 1497 mg/L, respectively). Possible reasons for the elevated SAA concentration in these clinically healthy foals include inflammation of noninfectious or nonsystemic origin (e.g. injury, meconium colic, birth trauma, local infection) (Chavette et al., 1992; Ludwig et al., 2016; Stoneham et al., 2001) or simply incipient infection prior to the onset of clinical signs. In such cases, SAA values >100 mg/L in neonatal foals are highly suggestive of infection and should assume the presence of an acute-phase response (Stoneham et al., 2001). Such cases warrant a SAA re-test within 24 h after the initial SAA test, pathogen-specific diagnostic testing and close monitoring as indicated in **Fig 2**. Serial SAA testing is advisable in cases where clinical evidence is ambiguous, in endemic settings, and to determine whether results indicate an increasing or declining SAA concentration.

Our results in foals were consistent with those in a previous study involving a large, diverse population of adult horses presented at an equine specialty practice (Belgrave et al.,

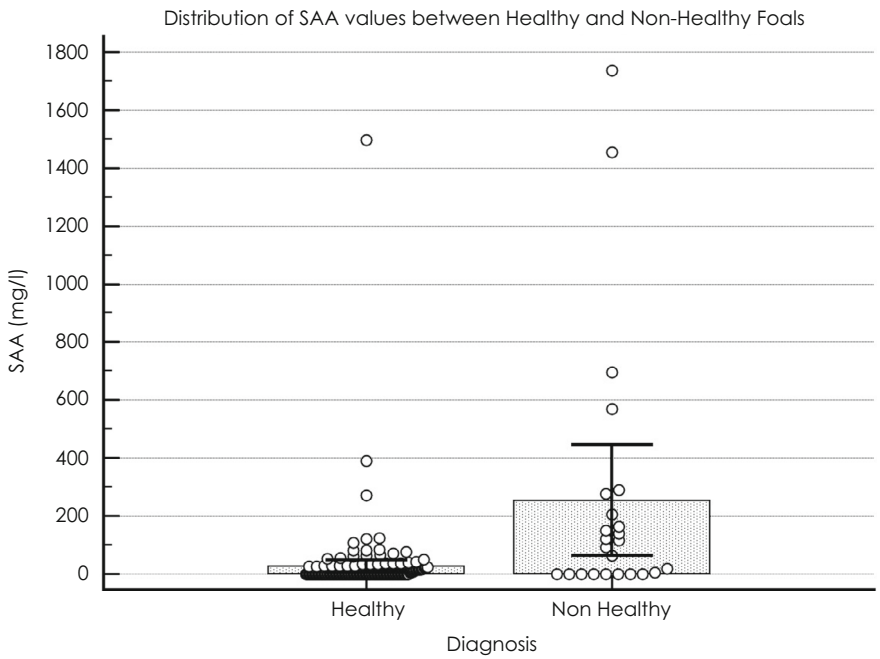

Fig 1: The figure shows the distribution of serum amyloid A (SAA) concentrations for a population of healthy (n = 151) and nonhealthy (n = 24) newborn foals. A SAA value of ≥100 mg/L was used as the threshold for a positive diagnosis. The mean SAA value was 26.6 ± 128.5 (range, 0–1497) mg/L in healthy foals and 253.9 ± 452.3 (range, 0–1737) mg/L in nonhealthy foals, a ninefold difference.

TABLE 2: Sensitivity and specificity diagnostic outcomes for serum amyloid A (SAA), immunoglobulin G (IgG) and white blood cell (WBC) tests in clinically healthy and nonhealthy newborn foals

	SAA test (100 mg/L)			IgG test (>8.0 g/L)			WBC test (5–12 × 10^3/μl)		
Test parameter	No. healthy foals	No. nonhealthy foals	Total no. foals	No. healthy foals	No. nonhealthy foals	Total no. foals	No. healthy foals	No. nonhealthy foals	Total no. foals
Positive test result	8	10	18	10	2	12	33	10	43
Negative test result	143	14	157	141	22	163	118	14	132
Totals	151	24	175	151	24	175	151	24	175
Sensitivity	41.7% (14/24)			8.3% (2/24)			41.7% (14/24)		
Specificity	94.7% (143/151)			93.4% (141/151)			78.1% (118/151)		

TABLE 3: Comparison of predictive values and test accuracy for acute-phase infectious disease in newborn foals based on serum amyloid A (SAA) and immunoglobulin G (IgG) concentrations and white blood cell (WBC) counts

Diagnostic test and normal range	Positive predictive value	Negative predictive value	Sensitivity	Specificity	Test accuracy
SAA <100 mg/L	55.6%	91.1%	41.7%	94.7%	87.4%
IgG >8.0 g/L	16.7%	86.5%	8.3%	93.4%	81.7%
WBC 5–12 × 10^9/L	23.5%	89.4%	41.7%	78.1%	73.1%
SAA + WBC	66.7%	88.2%	16.7%	98.7%	87.4%

2013). The investigators compared WBC, SAA and fibrinogen concentrations for clinically normal and clinically sick horses (n = 111 and 101, respectively). Using a laboratory turbidometric immunoassay with a 20 mg/L threshold for positivity, SAA testing had a 53% sensitivity, 94% specificity and 75% test accuracy. Horses with SAA values >20 mg/L were significantly ($p < 0.001$) more likely to be clinically sick. Notably, SAA concentrations in several horses became abnormally high within 24 h before clinical signs emerged, demonstrating the sensitivity of SAA as a predictor of

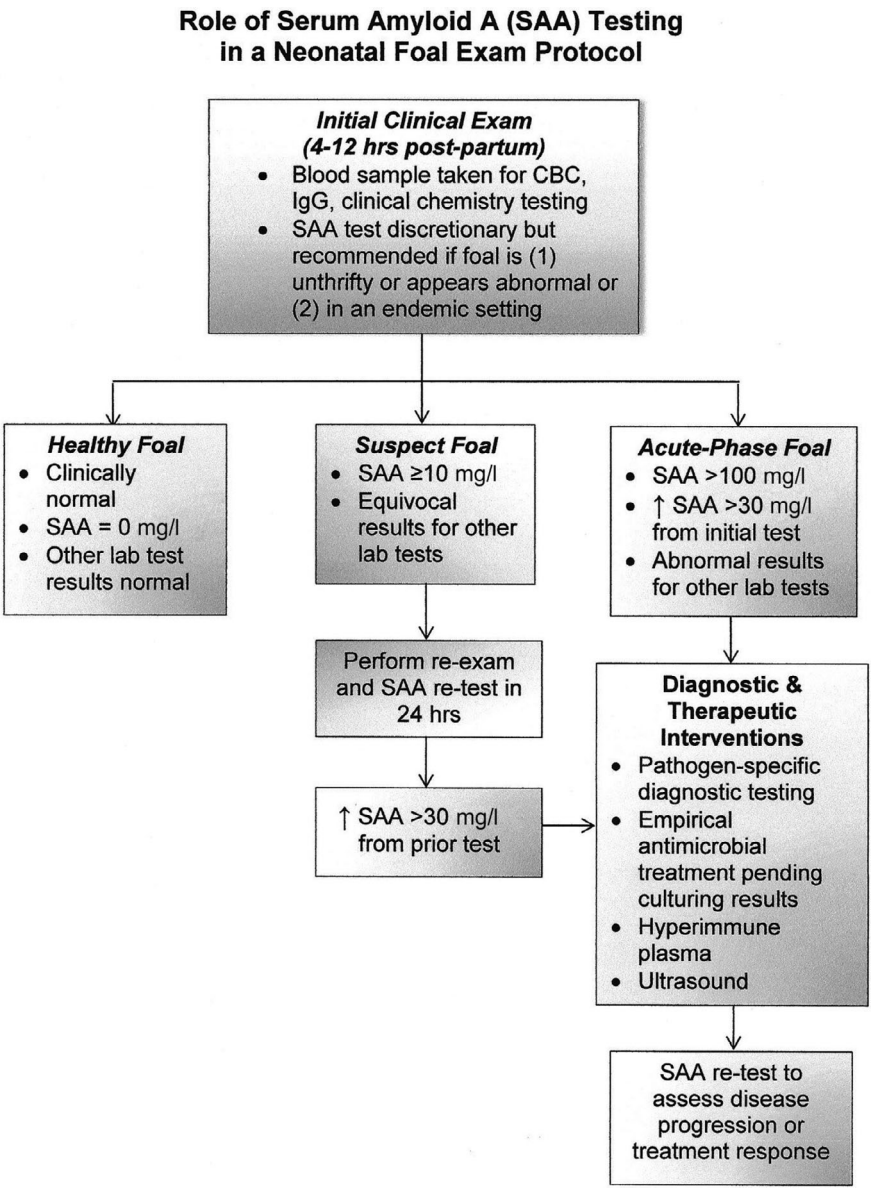

Fig 2: Serum amyloid A (SAA) testing assumed a prominent role in the neonatal foal examination protocol used at the study site. Because of its sensitivity as an acute-phase reactant and its broad dynamic range, SAA values were reliable interpretive indicators of subclinical or clinical disease, disease progression and response to treatment. Based on these outcomes, SAA testing was useful in categorising foals as healthy, suspect or acute-phase.

preclinical acute-phase infections. The authors found that SAA results do not necessarily correlate with WBC counts and fibrinogen concentration in clinically sick horses because of the varying kinetics involved. They concluded that SAA concentration was a more reliable indicator of inflammation or infection and return to health than either WBC or fibrinogen concentrations. Similarly, in a recent study comparing 54 pneumonic and 44 clinically healthy foals 3–5 months of age on a *R. equi* endemic farm, SAA concentrations were significantly higher in pneumonic versus normal foals (Giguere et al., 2016). At a SAA diagnostic threshold of 50 mg/L, SAA had a 57% sensitivity and 86% specificity, similar to the outcomes in our study using a 100 mg/L cut-off.

Various studies have evaluated the usefulness of SAA as a primary indicator of acute-phase respiratory disease, especially those caused by *R. equi* (Giguere et al., 2016; McCracken, 2019; Passamonti et al., 2015; Viner et al., 2017). Reports of SAA response in horses with enteritis, the most frequent presentation in sick foals in our study, are less common. A European study found that SAA values were not as definitive in foals presenting with mild or asymptomatic rotavirus diarrhoea versus those with pneumonia. The authors nevertheless concluded that SAA was still diagnostically useful, particularly in monitoring treatment response (Hulten & Demmers, 2002). Using SAA testing to assess treatment response or disease progression was not a component of our study, but would be a useful objective for future investigation,

particularly for evaluating SAA response to specific therapies or disease entities.

To date, SAA is the only major APP identified in the horse (Belgrave et al., 2013; Jacobsen & Andersen, 2007; Long & Nolen-Walston, 2020). Serum amyloid A has been demonstrated to be a much more sensitive and responsive biomarker of inflammation than other APP reactants widely used in human and veterinary clinical medicine, such as fibrinogen and C-reactive protein (Belgrave et al., 2013; Hulten & Demmers, 2002; Jacobsen & Anderson, 2007; Nunokawa et al., 1993). For example, fibrinogen concentrations in horses increase only modestly (twofold to fourfold) from constitutive levels during an acute-phase episode, with a relatively slow response time and elevated concentrations resolving gradually over several weeks (Jacobsen & Andersen, 2007). As such, fibrinogen is a minor APP that can be considered a lagging indicator of acute-phase infection and of limited value in directing prompt therapeutic intervention. In contrast, SAA concentrations can increase 10- to 100-fold or more from undetectable basal concentration during an acute-phase response, beginning within 6–12 h, peaking at 48 h and declining to normal concentration within hours after synthesis has stopped. This dynamic acute-phase response pattern allows SAA to function as a practical interpretive marker of preclinical inflammation, acute-phase progression and treatment response.

An acute-phase SAA response has been demonstrated in a wide variety of equine infectious diseases, including viral and bacterial respiratory and enteric infections, reproductive infections, septic arthritis and septicaemia (Belgrave et al., 2013; Giguere et al., 2016; Hulten & Demmers, 2002; Jacobsen & Anderson, 2007; Ludwig et al., 2016; Stoneham et al., 2001; Viner et al., 2017). Synovial fluid SAA concentrations have been shown to increase significantly in cases of septic arthritis (Ludwig et al., 2016). In addition to neonatal foal examination protocols, other applications of SAA testing include evaluating horses of unknown health status prior to commingling with a herd, monitoring spread of infectious disease within a herd as a basis for biosecurity mitigation, helping determine when a convalescent horse can return to training and assessing the health status of horses before or after shipping or performance.

A recent study on a Thoroughbred farm endemic for *Rhodococcus equi* illustrates how SAA can be effectively used for early recognition and appropriate treatment of infectious disease in foals, potentially minimising disease impact and therapeutic costs (McCracken, 2019). The investigator routinely used thoracic ultrasound screening of foals to identify subclinical pulmonary infection caused by *R. equi*, widely considered to be the most important and common cause of pneumonia in foals <6 months of age (Cohen et al., 2005; Giguere et al., 2016; Passamonti et al., 2015). Thoracic ultrasound screening on this farm was very effective in identifying even low-grade, subclinical *R. equi* lesions, cases which were then aggressively treated with antimicrobials. However, data showed when *R. equi*-infected foals were not treated, the great majority would recover without progressing to clinical disease. This finding meant that significant antimicrobial overtreatment with questionable benefit was occurring on the farm. When SAA testing was performed in the study population in conjunction with thoracic ultrasound, the positive predictive value for subclinical pulmonary infection to progress to clinical pneumonia increased threefold, from 17% for ultrasonography alone to 50% for ultrasonography plus SAA testing (McCracken, 2019). Treatment protocols on this farm were subsequently modified to greatly reduce antimicrobial overtreatment to levels consistent with judicious use. The study was an affirmation of the value of SAA in properly identifying at-risk animals with subclinical or early-onset infection, one of the most important diagnostic challenges facing equine practitioners.

Our study was designed to conform to circumstances typically encountered in clinical practice. As such, we used previously validated point-of-care diagnostic tests, and control samples or secondary testing were not employed as part of the study design. The clinical examination was the principal evaluative indicator of health status in this study, with point-of-care testing, including SAA, used in a secondary role to further define the clinical presentation. Stated another way, elevated SAA is generally not the most critical diagnostic biomarker, but an early, nonspecific indicator that an inflammatory process is going on, usually but not always an infection, indicating that close monitoring and additional diagnostic testing are warranted.

A neonatal examination protocol (**Fig 2**) illustrates how SAA testing can be effectively used for assessing the health status of foals and categorising them as clinically healthy, suspect or acute-phase patients. Implicit in the protocol are two 'best practices' for effectively using SAA testing in foals: a multimodal approach using other diagnostic methods and repeat testing in the case of suspect, acute-phase or convalescent patients. In other words, no single type of nonspecific test should be considered by itself, and repeat testing will indicate disease progression and treatment response. Because of the speed and convenience of using the point-of-care device described in this report, practitioners can readily perform serial SAA tests in ambiguous cases or where monitoring is advisable. Using the principles of repeat SAA testing in conjunction with other nonspecific screening methods, practitioners can have a high degree of confidence in the reliability of SAA testing as a diagnostic tool in equine practice, particularly for early detection of acute-phase infection.

Acknowledgement

The authors acknowledge the contributions of Mark Dana of Scientific Communications Services, LLC, in the writing and editing of the manuscript.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

No approval was needed for ethical review for this study because the study was based on additional testing of blood samples that were already taken on newborn foals as part of routine screening.

Informed consent

Informed consent was given.

Source of funding

The study was funded by Epona Biotech, Sligo, Ireland.

Authorship

N. Nieman was responsible for study design, study execution and final approval of the manuscript. D.-S. Chan was responsible for data analysis and interpretation.

References

Baratloo, A., Hosseinim, M., Negida, A. and El Ashal, G. (2015) Part 1: simple definition and calculation of accuracy, sensitivity and specificity. *Emergency* **3**, 48-49.

Belgrave, R.L., Dickey, M.M., Arheart, K.L. and Cray, C. (2013) Assessment of serum amyloid A testing of horses and its clinical application in a specialized equine practice. *Journal of the American Veterinary Medical Association* **243**, 113-119.

Chavatte, P.M., Pepys, M.B., Roberts, B., Ousey, J.C., McGladdery, A.J. and Rossdale, P.D. (1992) Measurement of serum amyloid A protein (SAA) as an aid to differential diagnosis of infection in newborn foals. *Equine Infectious Diseases* **6**, 33-38.

Cohen, N.D., Chaffin, M.K., Vandenplas, M.L., Edwards, R.F., Nevill, M., Moore, J.N. and et al. (2005) Study of serum amyloid A concentrations as a means of achieving early diagnosis of *Rhodococcus equi* pneumonia. *Equine Veterinary Journal* **37**, 212-216.

Giguère, S., Berghaus, L.J. and Miller, C.D. (2016) Clinical assessment of a point-of-care serum amyloid A assay in foals with bronchopneumonia. *Journal of Veterinary Internal Medicine* **30**, 1338-1343.

Giguère, S., Hernandez, J., Gaskin, J., Miller, C. and Bowman, J.L. (2003) Evaluation of white blood cell concentration, plasma fibrinogen concentration, and an agar gel immunodiffusion test for early identification of foals with *Rhodococcus equi* pneumonia. *Journal of the American Veterinary Medical Association* **222**, 775-781.

Hulten, C. and Demmers, S. (2002) Serum amyloid A (SAA) as an aid in the management of infectious disease in the foal: comparison with total leukocyte count, neutrophil count and fibrinogen. *Equine Veterinary Journal* **34**, 693-698.

Jacobsen, S. and Anderson, P.H. (2007) The acute phase protein serum amyloid A (SAA) as a marker of inflammation in horses. *Equine Veterinary Education* **19**, 38-46.

Long, A. and Nolen-Walston, R. (2020) Equine inflammatory markers in the twenty-first century: A focus on serum amyloid A. *The Veterinary Clinics of North America. Equine Practice* **36**, 147-160.

Ludwig, E.K., Wiese, R.B., Graham, M.R., Settlage, J.M., Werrre, S.R., Petersson-Wolfe, C.S. et al. (2016) Serum and synovial fluid serum amyloid A response in equine models of synovitis and septic arthritis. *Veterinary Surgery* **45**, 859-867.

McCracken, J.L. (2019) Evaluation of white blood cell, fibrinogen, serum amyloid A, and ultrasonographic grade to refine a *R. equi* screening program. *Proceedings of the American Association of Equine Practitioners* **65**, 522-530.

Nunokawa, Y., Fujinaga, T., Taira, T., Okumura, M., Yamashita, K., Tsunoda, N. et al. (1993) Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. *Journal of Veterinary Medical Science* **55**, 1011-1016.

Passamonti, F., Vardi, D.M., Stefanetti, V., Marenzoni, M.L., Prato, S., Cevese, P. et al. (2015) *Rhodococcus equi* pneumonia in foals: an assessment of the early diagnostic value of serum amyloid A and plasma fibrinogen concentrations in equine clinical practice. *The Veterinary Journal* **203**, 211-218.

Pepys, M.B., Baltz, M.L., Tennent, G.A., Kent, J., Ousey, J. and Rossdale, P.D. (1989) Serum amyloid A protein (SAA) in horses: objective measurement of the acute phase response. *Equine Veterinary Journal* **21**, 106-109.

Stoneham, S.J., Cash, P.R. and Rossdale, P.D. (2001) Measurement of serum amyloid A in the neonatal foal using a latex agglutination immunoturbidimetric assay: determination of the normal range, variation with age and response to disease. *Equine Veterinary Journal* **33**, 599-603.

Viner, M., Mazan, M., Bedenice, D., Mapes, S. and Pusterla, N. (2017) Comparison of Serum amyloid A in horses with infectious and noninfectious respiratory diseases. *Journal of Equine Veterinary Science* **49**, 11-13.