Serum amyloid A response post laparotomy in colic horses

Eva-Christina Schliewert, Lisanne Damhuis and Jan-Hein Swagemakers

Tierklinik Lüsche GmbH, Bakum, Germany

Summary: The acute phase protein serum amyloid A (SAA) has become widely used as a diagnostic aid for the early detection of inflammation. SAA increases by more than 25% in 24 to 38 hours after tissue damage caused by trauma or infection. To determine the increase in serum amyloid A (SAA) following colic surgery and to compare it to changes in leukocyte count, client owned horses > 2 years of age, presented for colic with emergency laparotomy were used. Whole blood SAA concentration and leukocyte count were determined before surgery and at 48, 72, and 96 hours post-surgery. Lesions were classified as non-inflammatory or inflammatory during surgery. 61 horses underwent emergency laparotomy; 44 horses were excluded. Of the remaining 17 horses, 12 had non-inflammatory, 5 inflammatory lesions. Before surgery SAA concentrations in horses classified as non-inflammatory ranged from 0 to 217 μg/mL (median 14 μg/mL, range 10–18) and from 0 to 987 μg/mL (median 162 μg/mL, range 162) in inflammatory cases. In all horses SAA concentrations increased significantly 48 hours post-surgery (p = 0.002), horses with inflammatory lesions had significantly higher SAA concentrations (median 2750 μg/mL, range 1916–2835) compared to non-inflammatory lesions (median 624 μg/mL, range 471–740) at 48 (p = 0.0004), 72 (p = 0.0009), and 96 (p = 0.0007) hours post-surgery. Maximum SAA concentration was observed 48–72 hours post-surgery in all cases. White blood cell count (WBC) revealed a mild decrease post-surgically in some cases. At discharge all horses had SAA concentrations < 200 μg/mL. Differences in SAA concentrations between inflammatory and non-inflammatory cases can be expected. The knowledge of normal postoperative SAA concentrations is essential if SAA is used for monitoring occurrence of postoperative infections.

Keywords: equine, colic, laparotomy, serum amyloid A, acute phase protein, acute phase response, inflammation

Correspondence: Dr. Eva-Christina Schliewert, Senior Lecturer in Equine Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa; tine.schliewert@web.de

Introduction

Over the last few years, serum amyloid A (SAA) has become widely used as a diagnostic aid for the early detection of inflammation. SAA is a major acute phase protein that increases by more than 25% in 24 to 38 hours after tissue damage caused by trauma or infection. Increases in whole blood SAA concentrations are considered a useful diagnostic indicator for post-surgical inflammation. Surgical incisions also result in an increase in whole blood SAA concentration (*Pepys* et al. 1989, *Hultén* et al. 1999, *Jacobsen* et al. 2005, *Jacobsen* et al. 2009). Alterations in leukocyte count also occur with infections but the sensitivity of leukopenia or leukocytosis may be lower than that of SAA (*Jacobsen* et al. 2009). Previous studies also reported that SAA concentrations are higher in horses with inflammatory causes of gastrointestinal tract disease compared with non-inflammatory causes of colic (*Vandenplas* et al. 2005, *Pihl* et al. 2015). Currently, only limited information is available on the magnitude of the SAA increase after laparotomy in colic horses, making the use of SAA to distinguish between physiological response and post-surgical inflammation caused by infection or exaggerated inflammatory response difficult.

The aims of this study were to determine the increase in whole blood SAA following colic surgery, evaluate potential differences in whole blood SAA in colics of inflammatory and non-inflammatory etiologies and non-survivors, to compare changes in SAA to changes in white blood cell count (WBC), and to establish the diagnostic value of SAA in horses post emergency laparotomy.

Methods

Horses presented with colic to the Tierklinik Lüsche from February 2016 to May 2017 requiring laparotomy (within 24 hours of admission) were included in the study. Prior to surgery, horses underwent a diagnostic evaluation including a physical examination, rectal palpation, nasogastric intubation, transabdominal ultrasonography, abdominocentesis, and fecal analysis for the presence of sand or parasite eggs. Laboratory blood testing upon admission included hematology and blood and (when possible) peritoneal fluid lactate concentration. Surgery indications included intractable pain or findings on examination that supported suspicion of a surgical gastrointestinal lesion. A final diagnosis was made during laparotomy; colic causes were classified as inflammatory with findings such as enteritis, local hyperemia and edema, ischemic lesions (with expected reperfusion injury) or severe strictures and scarring, while horses with simple large intestinal displacements (right dorsal displacement, left dorsal displacement or colonic torsions < 360°) or large intestinal impactions (pelvic flexure impaction) were categorized as non-inflam-

matory. Horses were excluded if they were < 2 years old, if they were pregnant > 7 months, or if an inflammatory disease unrelated to the abdomen was identified during the clinical examination (examples: respiratory infections, hoof abscesses, lacerations) as these might influence SAA concentrations. Horses were also excluded if repeat laparotomy was required. Blood was collected into EDTA tubes by venipuncture from a jugular vein or through the intravenous catheter immediately before and 48, 72, and 96 hours after surgery, and then at individual time points as determined by the attending clinician after this time. Owner informed consent was obtained prior to study enrollment for all horses.

Laboratory Analyses

Whole blood SAA concentration was measured using a handheld lateral flow assay (StableLab®, Epona, Sligo, Ireland) validated for equine use (Schwartz 2016), white blood cell count was determined with an automated hematology analyzer (Idexx Procyte Dx®, Idexx Laboratories, Ludwigsburg, Germany). Reference range for this analyzer is 0–15µg/mL.

Statistical Analysis

Statistical analysis was performed using a computer software program (Microsoft Excel, Redmont, WA, USA). Data distribution was checked for normality. As data was not normally distributed, median and interquartile range were reported. Statistical comparisons were performed using Kruskal-Wallis test to assess the effect of group on the SAA and WBC concentration. Sensitivity and specificity, positive and negative predictive values were calculated. Significance was set at $p < 0.05$.

Results

During the study period 61 horses that presented for colic underwent emergency laparotomy. 44 horses were excluded (31 were euthanized intraoperatively or within 5 days postoperatively due to poor prognosis or recurring colic, three were foals, one horse had a caesarian section, one horse underwent repeat laparotomy 48 hours after the initial surgery,

8 horses had incomplete records or missing samples). The 17 remaining horses had a median age of 12 years (range 9–16 years). 5 geldings and 12 mares were included. Thirteen horses were Warmbloods, 2 Icelandic horses, 1 draft horse and 1 pony. Of the 17 horses, 12 had non-inflammatory lesions such as simple displacements of the large intestine and/or impactions (3 horses were diagnosed with right dorsal displacement of the colon and impaction of the pelvic flexure, 1 horse with left dorsal displacement and impaction of the pelvic flexure, 7 horses were diagnosed with colon torsions < 360°), 5 horses had lesions considered to be of inflammatory origin with potentially infectious causes of unknown etiology (such as anterior enteritis, colitis).

Before surgery, the median SAA concentration was 5µg/mL (range 0–162), 14µg/mL (range 10–18) in the non-inflammatory and 162µg/mL (range 0–162) in the inflammatory group. Horses euthanized in surgery or in the first 96 hours post-surgery (pre-surgery sample available for n = 16) had a median SAA concentration of 67µg/mL (range 8–319). There was no significant difference in SAA concentration between survivors and non-survivors (p = 0.6703). (Figure 1, 2)

SAA concentration increased in all cases over the following sampling time points: at 48 hours post-surgery, the median SAA concentration was 739µg/mL (range 0–162), 624µg/mL (range 471–740) in the non-inflammatory and 2750µg/mL (range 1916–2835) in the inflammatory group. 72 hours post-surgery, the median SAA concentration was 666µg/mL (range 421–1124), 591µg/mL (range 253–697) in the non-inflammatory and 2772µg/mL (range 1911–3000) in the inflammatory group. 96 hours post-surgery, the median SAA concentration was 498µg/mL (range 255–1147), 447µg/mL (range174–512) in the non-inflammatory and 1817µg/mL (range 1657–2056) in the inflammatory group. SAA concentrations were not significantly different between the two groups before surgery (p = 0.37) but differed significantly at 48 hours (p = 0.001), at 72 hours (p = 0.001), and at 96 hours (p = 0.001) post-surgery. At the time of discharge (day 7 to 12 post-surgery) all horses had SAA concentrations < 200µg/mL.

WBC obtained simultaneously with SAA concentrations revealed a decrease 48 hours post-surgery in 14/16 cases.
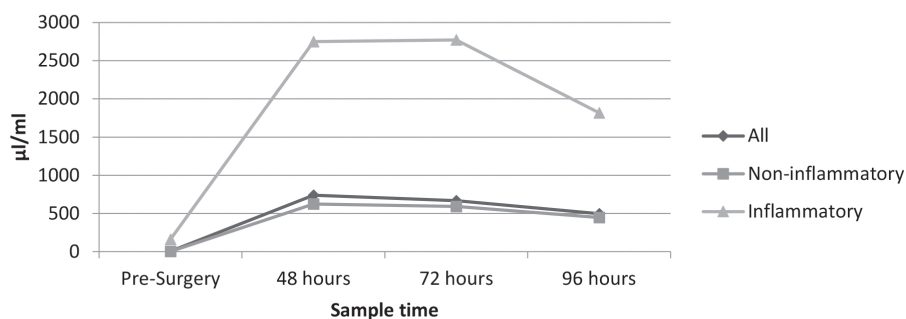

Fig. 1 Blood SAA-concentrations (µg/mL) taken from 17 horses undergoing emergency laparotomy for colic signs, before and 48h, 72h, and 96h post-surgery. Median SAA concentration for each time point is shown for: all 17 horses (blue circle), the 12/17 horses classified with a non-inflammatory surgical lesion during surgery (green triangle), and the 5/17 horses classified with an inflammatory surgical lesion (red square). A significant increase in blood SAA concentration in the inflammatory surgical lesion colic horse group compared to the non-inflammatory surgical lesion group is notable. |

SAA-Konzentration (µl/ml) von 17 Pferden mit Koliksymptomatik, bevor und 48h, 72h und 96h nach Laparotomie. Markiert ist die mittlere SAA-Konzentration für die verschiedenen Zeitpunkte bei allen 17 Pferden (blauer Kreis), 12/17 Pferden mit nichtentzündlichen Läsionen (grünes Dreieck) und 5/17 Pferden mit entzündlichen Läsionen. Ein signifikanter Anstieg in SAA ist in der Gruppe mit entzündlichen Läsionen erkennbar (verglichen mit nicht-entzündlichen Läsionen).

Seven horses (1 from the inflammatory, 6 from the non-inflammatory group) decreased to values below the reference range, but the decrease in WBC was not statistically significant (p = 0.14) in any group at any time point. Prior to discharge the WBC was within laboratory reference ranges in all cases.

WBC in horses euthanatized in surgery or in the first 96 hours post-surgery (pre-surgery sample available for n = 16) was not significantly different from WBC in survivors (p = 0.6102). Sensitivity and specificity were calculated for different SAA concentrations. On a receiver-operating-characteristics (ROC) curve, a cut off value of 700 µg/mL was identified to have a sensitivity of 100 % and a specificity of 92.3 % for an inflammatory lesion. The positive predictive value for an inflammatory lesion was 83.3, the negative predictive value 100 with an accuracy of 94.4 %.

Compared to a population of colic horses that were euthanatized intraoperatively (n = 7) or within the first 48 hours post-surgery (n = 3), SAA values were not significantly different before surgery (p = 0.543) or at any time point after surgery.

Discussion

The main goal of this study was to obtain information about the magnitude of the whole blood SAA response following emergency laparotomy. Expectedly, SAA increased significantly following emergency laparotomy with the maximum response occurring around 48 to 72 hours post-surgery. The increase in whole blood SAA was attributed mainly to surgical trauma. Surgical incisions (*Jacobsen* et al. 2009) and handling and manipulation of the intestines (*Schwarz* et al. 2004, *Hopster-Iversen* et al. 2011) are known to be associated with inflammation. The values obtained are higher compared to values reported for horses undergoing laparotomy for ovariectomy (*Jacobsen* et al. 2009) but lower than in horses undergoing emergency abdominal surgery (*Daniel* et al. 2016). The different magnitude of the SAA response is likely attributed to concurrent gastrointestinal inflammation occurring in the colic cases and seems to be also related to the extent of inflamed tissue as the underlying disease process (e.g. small intestinal versus large intestinal disease) likely determines magnitude of the acute phase response. Surgical technique

may also play an important role. As equine SAA has a half-life of 30–120 minutes (*Tape* et al. 1990), the delay of maximum SAA concentration until 48 to 72 hours post-surgery suggests continuous inflammation even after the primary surgical insult has occurred. Reperfusion injury following large colon ischemia has been shown to result in systemic inflammation (*Grosche* et al. 2013, *Daggett* et al. 2017), the increased SAA concentrations may also be influenced by this.

Contrary to previous reports (*Pihl* et al. 2016), we did not observe higher presurgical SAA concentrations in horses diagnosed with inflammatory lesions intraoperatively compared to horses with non-inflammatory lesions such as large colon displacements. Definitions for inflammatory lesions used by *Pihl* et al. were similar to the ones used in the present study but also included strangulating lesions with expected reperfusion injury causing inflammation. *Westermann* et al. described differences between horses with either medically managed or surgically corrected colic but did not further differentiate surgical findings. One reason for the different result between the present study and results from *Pihl* et al. may be that samples were taken in very early stages of the disease when SAA had not yet increased. 48 hours after surgery a highly significant difference in SAA between the two groups was observed. The exact reason for this difference is unknown but may be caused by additive effects on the SAA response by surgical trauma and – in horses in the inflammatory group – the underlying inflammatory pathology. The magnitude of the SAA response is probably directly related to the amount and extent of inflamed tissue so that inflammation of the gastrointestinal tract, which is observed in cases of enteritis or colitis (*Belgrave* et al. 2013, *Nemoto* et al. 2014), results in higher SAA concentrations. The correlation between SAA concentration and severity of colitis has been shown in mice (*De Villiers* et al 2000). Additionally, the difference between non-inflammatory and inflammatory may not always be obvious and misclassifications may have occurred. To the authors' knowledge, there is no published data evaluating SAA concentrations in specifically strangulating small intestinal lesions or inflammatory lesions such as colitis or enteritis. Due to the low numbers of horses included in this study, further differentiation was not possible. Further research on the magnitude of the SAA response in relation to the degree of inflammation is needed to better understand the inflammatory response.
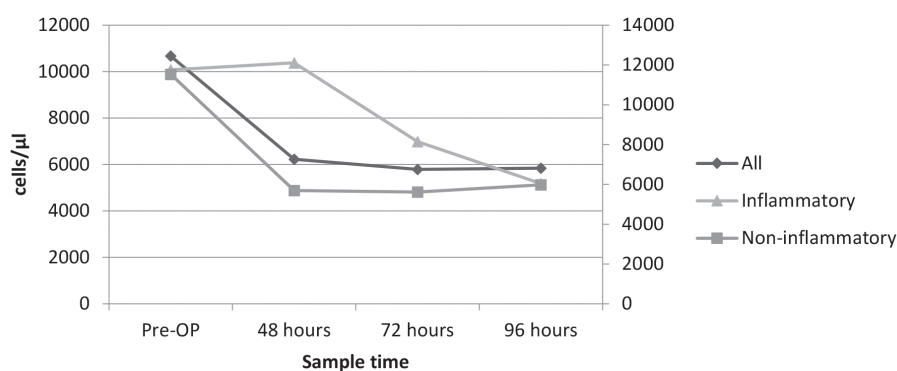

Fig. 2 WBC (cells/µL) from 17 horses undergoing emergency laparotomy for colic signs, before and 48 h, 72 h, and 96 h post-surgery. Median WBC for each time point is shown for: all 17 horses (blue circle), the 12/17 horses classified with a non-inflammatory surgical lesion (green triangle), and the 5/17 horses classified with an inflammatory surgical lesion (red square). An increase in WBC in the inflammatory lesion colic horse group (compared to the non-inflammatory surgical lesion group) is notable. | *WBC (cells/ µl) von 17 Pferden mit Koliksymptomatik, bevor und 48 h, 72 h und 96 h nach Laparotomie. Markiert ist der mittlere WBC-Wert für die verschiedenen Zeitpunkte bei allen 17 Pferden (blauer Kreis), 12/17 Pferden mit nichtentzündlichen Läsionen (grünes Dreieck) und 5/17 Pferden mit entzündlichen Läsionen. Ein Anstieg in SAA ist in der Gruppe mit entzündlichen Läsionen erkennbar (verglichen mit nicht-entzündlichen Läsionen).*

We expected a difference in the magnitude of SAA response between horses with uncomplicated post-surgical recovery and horses with complications such as post-operative ileus or incisional infection. SAA decreased in all but one case after 72 hours and decreased to values of $< 200\mu g/mL$ over the following days, consistent with clinical improvement. One horse had severely increased SAA concentrations before surgery, which continued to increase past the SAA reader's maximum detection limit of $3000\mu g/ml$ post-surgically. Intraoperatively, this horse was diagnosed with multiple inflammatory stenotic lesions and hyperemic lesions in the aboral half of the jejunum. SAA concentrations decreased rapidly following a change in antibiotic regime. Unfortunately, the owners declined jejunal biopsies and histopathological examination at the time of surgery and the underlying cause could not be identified. Two horses in the non-inflammatory group had a repeated increase in SAA after 72 hours and 96 hours respectively. These horses were diagnosed with mild incisional infections. SAA decreased with resolution of this infection after local lavage therapy was initiated. Post-operative ileus was observed in one horse, which had SAA levels of $> 2000\mu g/mL$ until the ileus resolved. Interestingly, WBC remained within reference ranges throughout this period. This emphasizes the superiority of SAA to detect acute inflammation (*Belgrave* et al. 2013, *Pollock* et al. 2005) but also the benefit of SAA monitoring in the post-operative phase to assist in the detection of non-resolving or new inflammatory foci, especially with repeated sampling. It is important to remember that an increase in SAA does not necessarily have to result directly from the surgical insult but may reflect secondary complications such as thrombophlebitis, POI, or incisional infections or even unrelated concurring disease processes such as infectious respiratory disease (*Viner* et al. 2016).

Some of the horses included had moderately increased SAA concentrations prior to surgery. This may have been caused by undetected previous inflammatory disease or the ongoing colic episode. *Pihl* (2015) reported that higher SAA concentrations at admission correlate with longer pre-admission duration of colic. The exact duration of colic was not known in most cases in the present study, but a correlation may have been present.

Unfortunately, the number of horses with inflammatory lesions was small and may not be representative, but the significant difference between the two groups indicates the presence of additional inflammation even in emergency laparotomies of non-inflammatory colic patients compared to elective procedures. Anesthesia alone has been shown to cause increases in SAA concentration.

Previous studies reported a decrease in WBC following colic surgery (*Salciccia* et al. 2013) and also observed a relationship between the magnitude of leukopenia and survival. A decrease was also observed in our population, eight horses developed leukopenia. Interestingly, at 48 and 72 hours following surgery in horses in the inflammatory group a mild increase in WBC was observed rather than a decrease. This was strongly influenced by one horse that developed severe leukocytosis after surgery and after excluding this horse, no obvious difference between the two groups was appreciable.

The point-of-care SAA reader used in this study has the advantage of delivering reliable results within ten minutes without the need for any advanced laboratory equipment. This allows for rapid decision-making and may instigate further diagnostic evaluation to identify new or non-resolving inflammatory foci.

This study had several limitations. The small number of horses in this study may have limited our ability to detect further differences between inflammatory and non-inflammatory types of colics. Unfortunately, many data sets were incomplete and resulted in exclusion. Furthermore, the number of horses euthanized intraoperatively is large. In many cases, owners elected exploratory laparotomy despite a poor prognosis for survival or because of insurance regulations. We also observed only a small number of complications (i.e. incisional infections, postoperative ileus) making it difficult to draw conclusions about the impact of these pathologies on the SAA concentration.

Diagnoses were made upon gross examination and surgical findings only, so it is possible that horses may have been unintentionally classified into the wrong category. Further histopathologic evaluation of tissues could have improved correct classification.

In retrospect, more frequent sampling, especially in the first hours after surgery, might have been beneficial to allow for better characterization of the SAA profile over time.

Given the findings of this study, SAA measurement before surgery and then every 48 hours is recommended to confirm decreasing concentrations. A persistently increased concentration or an increase in SAA are indicative of an ongoing inflammatory lesion and should result in further evaluation of the patient.

In conclusion, this study shows that SAA concentration increases post emergency laparotomy, the magnitude of the SAA response is likely related to the extent of tissue inflammation and the cause of the colic. Monitoring SAA concentrations post-surgery may help guide further diagnostic evaluation and patient management. SAA concentrations increase early in the inflammatory process and allow for adaptation of therapy, using a portable analyzer results are available immediately.

Conflict of interest

References

Aitken M. R., Stefanovski D., Southwood L. L. (2019) Serum amyloid A concentration in postoperative colic horses and its association with postoperative complications. Vet. Surg. 48, 143–151; DOI 10.1111/vsu.13133

Belgrave R. L., Dickey M. M., Arheart K. L., Cray C. (2013) Assessment of serum amyloid A testing of horses and its clinical application in a specialized equine practice. J. Am. Vet. Med. Assoc. 24, 113–119; DOI 10.2460/javma.243.1.113

Daggett J., Grosche A., Abbott J., Bauck A. J., McCarrel T. M., Graham S., Freeman D. E. (2017) Remote Responses To Intestinal Ischemia And Reperfusion Injury In Equine Colon And Jejunum. Equine Vet. Educ. 29, 27; DOI 10.1111/eve.58_12792

Daniel A. J., Leise B. S., Burgess B. A., Morley P. S., Cloninger M., Hassel D. M. (2016) Concentrations of serum amyloid A and plasma fibrinogen in horses undergoing emergency abdominal surgery. J. Vet. Emerg. Crit. Care 26, 344–351; DOI 10.1111/vec

De Cozar M., Sherlock C., Knowles E., Mair T. (2019) Serum amyloid A and plasma fibrinogen concentrations in horses following emergency exploratory celiotomy. Equine Vet. J. X; DOI 10.1111/evj.13117

De Villiers W. J. D., Varilek G. W., De Beer F. C., Guo J. T., Kindy M. S. (2000) Increased serum amyloid a levels reflect colitis severity and precede amyloid formation in il-2 knockout mice. Cytokine 12, 1337–1347

Grosche A., Morton A. J., Graham A. S., Polyak M. M., Freeman D. E. (2013) Effect of large colon ischemia and reperfusion on concentrations of calprotectin and other clinicopathologic variables in jugular and colonic venous blood in horses. Am. J. Vet. Res. 74, 1281–90; DOI 10.2460/ajvr.74.10.1281

Hopster-Iversen C., Hopster K., Staszyk C., Rohn K., Freeman D., Rötting A. K. (2011) Influence of mechanical manipulations on the local inflammatory reaction in the equine colon. Equine Vet. J. Suppl. 43, 1–7

Hultén C., Tulamo R. M., Suominen M. M., Burvall K., Marhaug G., Forsberg M. (1999) A non-competitive chemiluminescence enzyme immunoassay for the equine acute phase protein serum amyloid A (SAA) – A clinically useful inflammatory marker in the horse. Vet. Immunol. Immunopathol. 68, 267–281

Jacobsen S., Jensen J. C., Frei S., Jensen A. L., Thoefner M. B. (2005) Use of serum amyloid A and other acute phase reactants to monitor the inflammatory response after castration in horses: A field study. Equine Vet. J. 37, 552–556

Jacobsen S., Nielsen J. V., Kjelgaard-Hansen M., Toelboell T., Fjeldborg J., Halling-Thomsen M., Martinussen T., Thoefner M. B. (2009) Acute phase response to surgery of varying intensity in horses: A preliminary study. Vet Surg 38, 762–769; DOI 10.1111/j.1532-950X.2009.00564.x

Nemoto M., Oue Y., Morita Y., Kanno T., Kinoshita Y., Niwa H., Ueno T., Katayama Y., Bannai H., Tsujimura K., Yamanaka T., Kondo T. (2014) Experimental inoculation of equine coronavirus into Japanese draft horses. Arch. Virol. 159, 3329–3334; DOI 10.1007/s00705-014-2205-1

Pollock P. J., Prendergast M., Schumacher J., Bellenger C. R. (2005) Effects of surgery on the acute phase response in clinically normal and diseased horses. Vet. Rec. 156, 538–542

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

Pihl T. H., Scheepers E., Sanz M., Goddard A., Page P., Toft N., Andersen P. H., Jacobsen S. (2015) Influence of disease process and duration on acute phase proteins in serum and peritoneal fluid of horses with colic. J. Vet. Intern. Med. 29, 651–658; DOI 10.1111/jvim.12542

Salciccia A., Sandersen C., Grulke S., de la Rebière de Pouyade G., Caudron I., Serteyn D., Detilleux J. (2013) Sensitivity and specificity of blood leukocyte counts as an indicator of mortality in horses after colic surgery. Vet. Rec. 173, 267; DOI 10.1136/vr.101503

Schwartz D., Pusterla N., Christopher M. M. (2018) Analytical Validation of a new Point-of-Care Assay for Quantification of Serum Amyloid A in Horses. Equine Vet. J. 50 (5), 678–683; DOI 10.1111/evj.12807

Schwarz N. T., Kalff J. G., Turler A., Speidel N., Grandis J. R., Billiar T. R., Bauer A. J. (2004) Selective jejunal manipulation causes postoperative pan-enteric inflammation and dysmotility. Gastroenterology 126, 159–169; DOI 10.1053/j.gastro.2003.10.060

Tape C., Kisilevsky R. (1990) Apolipoprotein A-I and apolipoprotein SAA half-lives during acute inflammation and amyloidogenesis. Biochim. Biophys. Acta 1043, 295–300.

Vandenplas M. L., Moore J. N., Barton M. H., Roussel A. J., Cohen N. D. (2005) Concentrations of serum amyloid A and lipopolysaccharide-binding protein in horses with colic. Am. J. Vet. Res. 66, 1509–1516; DOI 10.1016/j.jevs.2016.09.005