EVALUATION OF HEMOSTATIC PARAMETERS AND SERUM AMYLOID (A) IN HORSES WITH ABDOMINAL PAIN

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ABSTRACT

The objective of the present study was to investigate the coagulation disorders and acute phase protein (serum amyloid A) in horses with abdominal pain. A total of 80 native breed horses of both sexes were examined. Based on the history, physical and laboratory investigations, the examined horses were classified into two groups: group 1 (n = 30): Healthy served as a control, and group 2 (n = 50) exhibited clinical signs of abdominal pain. Animals in group 2 were retrospectively subdivided into 3 subgroups: Diarrhea (n = 10), Impaction (n = 18) and Spasm (n = 22).The obtained results revealed that all horses with abdominal pain showed restlessness, sweating, lip curling, flank watching, pawing, rolling, and an increase in the heart and respiratory rate as well as capillary refilling time (CRT). On abdominal auscultation, horses with spasm and diarrhea had intestinal hypermotility while there was reduction in intestinal sounds in horses with impaction. Hematological examination showed subtle changes in hemogram and leukogram in diseased group. Hemostatic profile showed significant decrease (p<0.05) in platelet count (PLT$\times10^4$/ µl) in diarrheic and impacted groups (14.3 ± 1.35 and 14.9 ± 0.78, respectively) and significant increase (p<0.05) in prothrombin time (PT/sec) in spasmodic group (14.00± 0.75) and marginally increase in diarrheic and impacted groups (12.40 ± 0.57 and 12.70 ± 1.37, respectively). Additionally, activated partial thromboplastin time (APTT/sec) was significant increased (p<0.05) in spasmodic group (53.79 ± 2.44) and marginally increased in diarrheic and impacted group (48.3 ± 5.75 and 45.7 ± 4.13, respectively). Antithrombin III (AT III, mg/dl) was marginally decreased in horses suffering from diarrhea, impaction and spasm (29.0 ± 3.42, 29.7 ± 3.19 and 29.6 ± 2.73, respectively). Level of serum amyloid A (µg/ml) showed significant increase (p<0.05) in diarrheic and spasmodic groups (2.03 ± 0.50 and 1.6 ± 0.16, respectively), while marginally increase in impacted group (1.32 ± 0.09).

In conclusion, abdominal pain in horses induces changes in the coagulation system and acute phase protein (serum amyloid A). Serum amyloid A is a sensitive biomarker for abdominal pain in horses and could be used as diagnostic biomarker for equine colic.

Key words: equine, colic, hemostatis, serum amyloid A

INTRODUCTION

Equine colic is considered as the most universally experienced health problem and remained as the common cause of death in horses (Snyder and Spier, 1992).Horses with colic are generally exhibited signs of abdominal pain (Abutarbush *et al.,* 2005) including restlessness, kicking, rolling, sweating, getting up and lying down, flank watching and pawing (Alsaad and Nori, 2010). Horses are susceptible to a variety of gastrointestinal incidents, manifesting themselves asepisodes of abdominal pain or colic. Anatomical and epidemiological studies have identified several risk factors such as long gastrointestinal tract with marked variations in its diameter (White *et al.,* 2008), improper feeding strategy, changes in management practices and lack of water access (Kaneene *et al.,* 1997; Jennings *et al.,* 2014).

Hemostatis is a complex process with positive and negative regulators for formation or degradation of fibrin (Prasse *et al.,* 1993). Imbalanced regulation may lead to hyper-coagulation (thrombosis), hypo-coagulation (hemorrhage) or paradoxically to both of them (Byars, 1987; Collatos *et al.,* 1995; Petterson *et al.,* 1995). Hemostatic dysfunction is reported in colic (Monreal *et al.,* 2000). Systemic activation of the coagulation system associated with inflammation and/or endotoxemia may result in thrombocytopenia from platelet activation and consumption (Walton, 2012). Identification of platelet activation may allow early therapeutic intervention for prothrombotic status in colic patients with an inflammatory etiology (Hackner, 2011). Hemostatic alterations are expected outcomes in horses diagnosed with acute abdomen

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and probably associated with high rates of morbidity and mortality in those animals.

Recently, studies on acute phase proteins have drawn the attention of equine veterinary community for its ability to indicate inflammation (Anhold *et al.,* 2014). Serum amyloid A (SAA) has been identified as a major acute phase protein in humans and several veterinary species including horses (Hulteˊn and Demmers, 2002 and Jacobsen *et al.* (2004). It is produced in the liver as a part of body defense in response to stimuli including trauma, pain stress, infection, neoplasia and inflammation (Pihl *et al.,* 2013).

We propose that levels of hemostatic parameters and SAA can be useful as a diagnostic aid in equine colic. Therefore, the present study aimed to evaluate the serum amyloid A (SAA) as a major acute phase protein and hemostatic profile in terms of platelet count (PLT), prothrombin time(PT), activated partial thromboplastin time (APTT) and antithrombin III (AT III) in horses with abdominal pain.

MATERIALS ANDMETHODS

Animals: The study population included those equines admitted at the Veterinary Teaching Hospital (VTH), Faculty of Veterinary Medicine, Assiut University, Brooks Hospital and Animal Care Egypt (ACE) at Luxor Governorate. Total number of 80 animals of both sexes and different ages (2 -7 years) were included in the present study. These animals were classified into two main groups: Healthy one (n =30) served as a control and diseased one with abdominal pain (n=50). Diseased animals were retrospectively subdivided into 3 subgroups: diarrheic (n=10), impaction (n = 18) and spasm (n = 22).

Samples: Ten ml blood samples were collected from all animals by means of jugular vein puncture using 18G needle. Blood samples were distributed and used as following: 2.5ml of blood mixed with EDTA for complete blood count (CBC), 5ml of blood mixed with 3.8% sodium citrate (1 part citrate: 9 parts blood) used for collection of citrated plasma for determination of hemostatic parameters. 2.5ml blood without anticoagulant for serum collection for determination of serum amyloid A (SAA).

Reagent and kits: Thromborel®S, Pathromtin®SL and Berichrom*antithrombin III reagents were obtained from Siemens Healthcare Diagnostics Product (U.S.A) and used for determination of Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and functionally active Anti -Thrombin III (ATIII). ELISA kit for determination of serum amyloid A in horses was obtained from WKEA Med (China).

Adopted Methods
History and clinical examination: Animal's data and general examination including age, sex, duration of clinical signs, body temperature (^{o}C), respiratory rate (RC/min), heart rate (beats/min) and CRT (sec) were recorded. Physical examination was conducted according to Smith (2002).

Complete blood count (CBC) evaluation: Samples were analyzed directly after collection for CBC including total red blood cells (TRBCs$\times10^6$/μl)), hemoglobin (Hb, g/l), packed cell volume (PCV%), mean corpuscular volume (MCV/fl), mean corpuscular hemoglobin (MCH/pg) and mean corpuscular hemoglobin concentration (MCHC, g/dl), total white blood cells (TWBCs$\times10^3$/μl) and differential leukocytic count. Platelet enumeration (PLT$\times10^4$/μl) was also recorded on CBC report.

Determination of hemostatic parameters: Hemostatic assays were determined on citrated blood samples. The following parameters were determined.

Prothrombin time (PT/sec) was determined according to Poller, (1998) and CLSI (2008). Plasma PT level was determined on an automated analyzer using lyophilized human placental calcium thromboplastin (Thromborel®s reagent)as an activator of the extrinsic pathway of coagulation. The coagulation process is initiated by incubation of plasma with an optimal amount of thromboplastin and calcium for activation of extrinsic coagulation factors. The time to formation of fibrin clot is then measured. Briefly, 200 μl of Thromborel®s reagent was added to100 μl of citrated plasma and the coagulation time was determined using Thrombostat coagulometer (German manufacturer Behnk Elektronik).

Activated partial thromboplastin time (APTT/sec) is a screening test used to assess the intrinsic pathway of coagulation and was determined according to Thomson and Poller (1985). Briefly, 100 μl of citrated plasma sample was incubated with 100 μl of Pathromtin SL (phospholipids comprising partial thromboplastin and a surface activator silicon dioxide particles) to activate the intrinsic coagulation system. The addition of calcium ions (100μl of calcium chloride solution) triggers the coagulation process. The time to formation of fibrin clot is measured in second using Thrombostat coagulometer (German manufacturer Behnk Elektronik).

Antithrombin activity (ATIII, mg/dl): Anti-thrombin activity (AT III), physiological inhibitor of thrombin (Olson and Björk 1994), was determined with an automated analyzer using a chromogenic substrate test kit, Berichrom®Antithrombin III, at 37 °C according to Hathaway, (1991). The assay is based on the inhibition of thrombin by AT III and the residual thrombin content is determined kinetically using chromogenic substrate at absorbance 405 nm. Briefly, plasma sample was diluted with isotonic saline at 1:40. Fifty μL of diluted plasma sample was added to 1000 μL of thrombin reagent, thoroughly mixed and incubated for 3 minutes, then 100 μL of

substrate reagent (Berichrom®antithrombin III) was added and mixed. The amount of color released decreases linearly with the amount of antithrombin III present in the test sample. The absorbance was measured spectrophotometrically at 405nm.

Determination of serum amyloid A (µg/ml): SAA was determined according to Jacobsen and Anderson, (2007). ELISA test allows for *in vitro* quantitative determination of horse SAA concentrations in serum, plasma and other biological fluid. The kit assays SAA level in the tested sample using purified horse SAA antibody coated-microtiter plate wells. Briefly, 100 µL of serum sample was added to each well, mixed and incubated for 30 min at 37°C, washed and blotted dry 3 times. 100 µL of horse reddish peroxidase antibody conjugate reagent was added to each well, incubated for 30 min at 37°C, washed and blotted dry 3 times. 100 µL of substrate was added, and microtiter plate was incubated for 15 min at 37°C, substrate became blue color. The reaction was stopped using 100 µL sulphuric acid solution to each well. The optical density (OD) of each well was determined within 15 min by microtiter ELISA reader (Stat Fax 2100, Awareness, USA).

Statistical analysis: The obtained data were statistically analyzed using statistical software (GraphpadPrism 5). The mean value ± standard error of the mean (SE) was calculated. The significance of variations between control and diseased group was determined using T-test followed by Tukey's test.

RESULTS

History and clinical signs: Diseased horses were submitted with one or more of the following history: Eating large amount of grains, green fodders and rough dry fodder then stop eating, no defecation and/or diarrhea, anorexia and lying down. Initial assessment of submitted cases showed sign of pain (Figure 1a) including pawing, anxiety, and rolling on the ground. Submitted cases were classified according to etiology of abdominal pain into diarrhea (n=10), impaction (n=18) and spasm (n=22). The mean values

($\pm$SE) of body temperature (^{o}C), respiratory rate (RC/min), heart rate (beats/min) and CRT (sec) in horse with diarrhea, impaction and spasm were presented in Table 1. Auscultation of the four quadrates of abdomen revealed loud intestinal sounds in spasm while there was marked reduction in the intestinal sounds in case of impaction with slight abdominal distention (Figure 1b). Rectal examination revealed no obvious abnormalities in spasm while there was hard material at the ventral colon in impaction.

Laboratory findings: Hematological analysis in healthy and diseased horses revealed marginally increase in different groups of abdominal pain, but not statistically difference (Table 2) and marginally decrease in TWBCs in colicky horses suffered from diarrhea, impaction and spasm with no variation in differential leukocytic count.

Results of hemostatic parameters in healthy and diseased animals were presented in Table 3 & Figure 2a-d.Platelet count (PLT) was significant decreased (p<0.05) in diarrheic and impacted groups while no statistically differences in spasmodic group (Figure 2a). Prothrombin time (PT) was significant increased (p<0.05) in spasmodic colic and marginally increased in impacted and diarrheic groups (Figure 2b). Additionally, activated partial thromboplastin time (APTT) was significant increased (p<0.01) in spasmodic group and marginally increased in impacted and diarrheic groups (Figure 2c). Determination of ATIII revealed marginally decrease in diarrheic, impacted and spasmodic groups (Figure 2d). SAA concentration in tested samples was determined by comparing the OD of the sample to the standard curve. Standard curve was prepared using various concentrations (15 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml) of SAA standard (Figure 3a). Results of serum amyloid A (SAA) in healthy and diseased horses were shown in Figure 3b. There were significant increased (p<0.05) in SAA in diarrheic and spasmodic groups, while marginally increased in impacted group.

Figure 1a: Four years old- mare suffered from abdominal pain

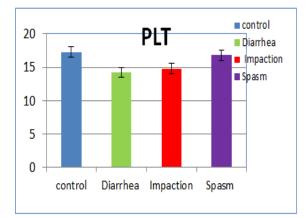**Figure 1b:** Four years old- mare showed abdominal distention

Figure 2a. Blood platelet count (PLTx10^4/ µl) in different groups of horses with abdominal pain
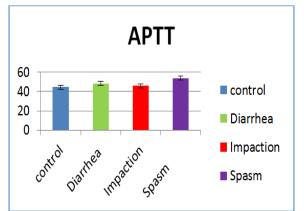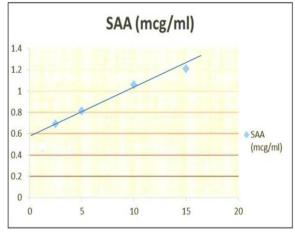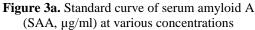

Figure 2.b. Prothrombine Time (PT/sec) in different groups of abdominal pain

Figure 2.c. Activated partial thromboplastin time (APTT/sec) of different groups of horses with abdominal pain
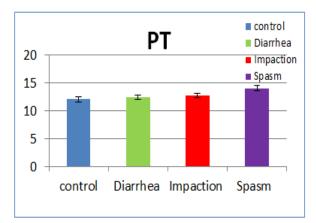

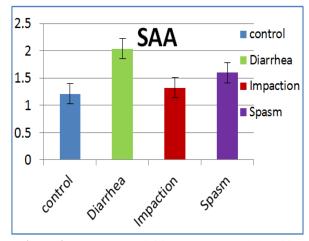**Figure 2.d.** Anti -Thrombin III (AT III, mg/dl) of different groups of horses with abdominal pain

Figure 3a. Standard curve of serum amyloid A (SAA, µg/ml) at various concentrations

Figure 3.b. Serum amyloid A (SAA, µg/ml) level in different groups of horses with abdominal pain

Table 1: Mean values (± SE) of body temperature, respiratory rate, heart rate and CRT in horses with diarrhea, impaction and spasm

Parameter	Control (n=30)	Abdominal Pain with Diarrhea (n=10)	Abdominal Pain with Impaction (n=18)	Abdominal Pain with Spasm (n=22)
Temperature (^{o}C)	37.5±0.10	38.5±0.28	37.7±0.18	38.3±0.28
Respiration rate (cycle/min)	17.0±0.58	18.3±1.81	17.1±0.66	20.2±0.69
Heart rate (beat/min)	40.56±0.51	41.3±1.48	42.7±1.79	48.2±1.95
Capillary refilling time (CRT/sec)	1.7±0.09	2.0±0.33	3.2±0.37	2.4±0.26

Table 2: Mean values (± SE) of hematological parameters of different groups of abdominal pain

Parameters	Control (n=30)	Abdominal pain associated with diarrhea (n=10)	Abdominal pain associated with impaction (n=18)	Abdominal pain associated with Spasm(n=22)
RBCs (×10^6/µl)	6.87 ± 0.24	6.78 ± 0.51	6.59 ± 0.319	7.02 ± 0.39
HB (g/dl)	11.59 ± 0.34	11.27 ± 0.61	11.69 ± 0.571	12.04 ± 0.59
PCV (%)	31.0 ± 0.80	34.49 ± 1.45	32.28 ± 1.41	34.17 ± 1.63
MCV (fl)	48.10±4.91	46.91 ± 1.94	46.06 ± 1.37	48.29 ± 1.36
MCH (pg)	17.13 ± 2.10	16.94 ± 0.68	17.71 ± 0.518	17.49 ± 0.63
MCHC (g/dl)	35.69 ± 3.55	36.23 ± 1.36	37.41 ± 0.97	36.24 ± 0.93
PLT (×10^4/µl)	17.3 ± 0 .78 [a]	14.3 ± 1.35 [b]	14.9 ± 0.78 [b]	16.85 ± 0.55 [a]
TWBCs (×10^3/µl)	12.88 ± 0.63	11.08 ± 1.38	10.66 ±1.05	11.86 ±1.19
Neutrophil (10^3/µl)	7.72 ± 0.65	6.84 ± 1.16	6.71 ± 1.17	6.99 ± 0.92
Lymphocytes (10^3/µl)	3.79 ± 0.23	3.08 ± 0.32	3.59 ± 0.39	3.48 ± 0.45
Monocytes (10^3/µl)	0.75 ± 0.06	0.9 ± 0.28	0.67 ± 0.115	0.65 ± 0.09
Eosinophil (10^3/µl)	0.04 ± 0.016	0.02 ± 0.015	0.03 ± 0.014	0.04 ± 0.029
Basophil (10^3/µl)	0.35 ± 0.055	0.21 ± 0.072	0.23 ± 0.054	0.43 ± 0.12

For each parameter, different letters indicate significant differences when compared with the control group.

Table 3: Mean values (± SE) of coagulation parameters in different groups of abdominal pain

Parameter	Control (n=30)	Abdominal pain associated with diarrhea (n=10)	Abdominal pain associated with impaction (n=18)	Abdominal pain associated with spasm (n=22)
PLT (×10^4/µl)	17.3 ± 0.78 [a]	14.3 ± 1.35 [b]	14.9 ± 0.78 [b]	16.85 ± 0.55 [a]
PT (sec)	11.9 ± 0.29 [a]	12.4 ± 0.57 [a]	12.7 ± 1.37 [a]	14.0 ± 0.75 [b]
APTT (sec)	44.32 ± 2.26 [a]	48.3 ± 5.75 [a]	45.7 ± 4.13 [a]	53.79 ± 2.44 [b]
AT III (mg/dl)	32.26 ± 1.84	29.0 ± 3.42	29.7 ± 3.19	29.6 ± 2.73

For each parameter, different letters indicate significant differences when compared with the control group.

DISCUSSION

Equine colic remained as the most common cause of death in horses and a major concern for all horse owners. During clinical examination, horses expressed signs of abdominal pain, kicking at the abdomen, lying down, rolling, pawing, sweating, stretching and reduced fecal output were recorded. These findings were in an agreement with those reported by Pratt *et al.* (2003); Alsaad and Nori, (2010) and Scantlebury *et al.* (2011). Abdominal pain could be attributed to severe intestinal contractions, inflammation, abdominal distension with over stretching of the intestine making severe stimulation

of nociceptors (Reeves *et al.,* 1989 and Radostits *et al.,* 2007).

Horses with abdominal pain due to diarrhea, impaction and spasm showed increase in the heart rate (41.3±1.48, 42.7±1.79 and 48.2±1.95 beats/min, respectively), respiratory rate (18.3±1.81, 17.1±0.66 and 20.2±0.69 RC/min, respectively) and in capillary refilling time (2.0±0.33, 3.2±0.37 and 2.4±0.26 CRT/sec, respectively). Abdominal auscultation revealed reduction in intestinal motility in case of impaction, while diarrheic and spasmodic groups showed audible sounds. These findings agreed with those reported by Thoefner *et al.* (2001); Hillyer *et al.* (2008), Bryan *et al.* (2009), Sutton *et al.* (2009);

Alsaad and Nori (2010) and Ibrahim, (2014). The increasing in heart rate, respiratory rate and CRT in affected horses could be associated with pain and poor peripheral perfusion.

Screening of the hematological data revealed no significant changes in the hemogram, however packed cell volume (PCV %) showed marginally increased, but not statistically difference. Slight increase in PCV could be attributed to some degree of dehydration and malabsorption which are common features in intestinal disturbances (Ayaz*et al.,* 1999; Radostits *et al.,* 2007 and Alsaad and Nori, 2010).While the short course of abdominal pain associated with spasm, diarrhea and impaction did not have major changes on the hemogram values; there was marginally decrease in TWBCs. This could be related to stress and abdominal discomfort (Lassen and Swardson, 1995; Moore and Palmer 2001; Radostits *et al.,* 2007).

The minimum laboratory data needed to evaluate hemostasis in equines are PLT, PT, APTT and AT III (Morris, 1990). Brooks (2008) reported that tests used to evaluate the hemostatic function can be classified as tests of primary (PLT count) and secondary hemostasis (PT, APTT and AT III). Evaluation of these indices are the most useful and practical because they assess the intrinsic and extrinsic pathway of coagulation process (Roberts *et al.,* 2006). In the current study, platelet count (PLT$\times10^{4}$/µl) revealed significant decrease (p<0.05) in diarrhea and impaction (14.3 ± 1.35 and 14.9 ± 0.78, respectively), and no statistical differences in spasm (16.85 ± 0.55). Thrombocytopenia could be attributed to platelet activation, consumption and depletion due to the release of endogenous mediators such as platelet activating factor in inflammatory disorders (Imaz *et al.,* 2002).Our results were in an agreement with Sandholm *et al.* (1995) and Monreal *et al.* (2000). Data of PT (sec) and APTT (sec) revealed significant increase in spasmodic colic (PT 14.0± 0.75 and APTT 53.79 ± 2.44) and marginally increased in impaction and diarrheic groups. These findings were in an agreement with Michelle and Moore (1991). Authors reported prolongation in PT and APTT were considered the most frequently observed abnormality in horses with colic. This could be explained as a result of decreased coagulation factors that is related to consumption coagulopathy of coagulation factors (Barton *et al.,* 1998 and Weiss and Rashid, 1998).

Antithrombin III (ATIII) is a regulator of thrombin and other procoagulant enzymes. It considered a major inhibitor of thrombin and other serine proteases of the coagulation system (Monreal *et al.,* 2000). In our study, values of AT III (mg/dl) revealed marginally decrease in diarrheic, impacted and spasmodic groups (29.0 ± 3.42, 29.7 ± 3.19 and 29.6 ± 2.73, respectively). These results consistent with those reported by other investigators (Morris, 1990;

Welch *et al.,* 1992 and Monreal *et al.,* 2000) which support the statement that decreased AT III activity is common in horses with gastrointestinal disorders. This result could be associated with depletion and consumption of procoagulant modulator ATIII in an acute consumptive coagulopathy (Darien *et al.,* 1989).

Zbanyszek *et al.* (2004) reported that colic in horses often alters the coagulation system, which promoted by an increase in exposition of coagulation activators with a simultaneous decrease in coagulation inhibitors activity, mainly antithrombin III. Coagulopathy with decreased procoagulant and profibrinolytic factors is common in horses with gastrointestinal disease (Monreal *et al.,* 2000, Dallap and Epstein, 2009 and Cesarini *et al.,* 2010). These changes are consistent with activation and subsequent consumption of coagulation factors. Künzer (1988) stated that consumption coagulopathy occurred due to imbalance or disturbance of blood clotting system which leading to an increased turnover of coagulation factors and platelets. The author added that after a period of hypercoagulability, hemostasis changes into hypo-coagulability due to deficiency of coagulation factors and platelets in circulation.

In this regards, horses with gastrointestinal disorders such as diarrhea, impaction and severe intestinal contraction as in spasm could be associated with endotoxin production. Endotoxin and tissue thromboplastin released from damaged gastro-intestinal tract may activate the extrinsic and intrinsic coagulation factors (Johnstone and Crane, 1986). The moderate thrombocytopenia and prolonged clotting times (PT and APTT) in horses were indicative of platelet/ clotting factor activation and consumption (Johnstone and Blackwell, 1984).

Moreover, horses with acute gastrointestinal disease induced an acute phase reaction with consecutive changes in the coagulation parameters mainly reflected by increased level of acute phase protein, serum amyloid A (Topper and Prasse, 1998 and Anhold *et al.,* 2014). Serum amyloid A (SAA) has become increasingly recognized by equine veterinary community for its ability to indicate early inflammation and identified as a major acute phase protein in horses (Anhold *et al.,* 2014 and El-Deeb *et al.,* 2016). In the present study, levels of SAA (µg/ml) showed significant increase (p<0.05) in diarrheic and spasmodic groups (2.035 ± 0.50 and 1.6 ± 0.16, respectively) and marginally increased in impacted group (1.32 ± 0.089). These results agreed with Nunokawa *et al.* (1993) and Vandenplas *et al.* (2005); Pihl *et al.* (2013) and Dondi *et al.* (2015). Pihl *et al.* (2013) stated that the concentration of SAA increased quickly in response to inflammation and tissue injury. Moreover, the authors confirmed increasing in SAA as a response happened in animals with conflicts in hemostasis and subsequent tissue injury.

CONCLUSION

Abdominal pain in horses due to diarrhea, spasm and impaction induces changes in the coagulation system (coagulopathy) in terms of thrombocytopenia, prolonged PT, APTT and decreased AT III. Serum amyloid A is a sensitive biomarker for abdominal pain in horses and could be used as diagnostic biomarker for equine colic.

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تقييم مؤشرات النزف ومصل الأميلويد (أ) في الخيول المصابة بآلام البطن

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تمت هذه الدراسة لتسليط الضوء على تأثير آلام البطن على مؤشرات النزف وبروتينات المرحلة الحادة (مصل الأميلويد- أ). ولتحقيق هذه الأهداف، تم فحص ثمانون (٨٠) من الخيول المحلية من كلا الجنسين. استناداً إلى التاريخ المرضى والفحوصات الاكلينيكية والمعملية لكل حيوان ، تم تقسيم الخيول إلى مجموعتين: المجموعة ١: الحيوانات السليمة (٣٠) بمثابة مجموعة ضابطة للحصول على المعدلات الطبيعية لمعاملات النزف، ومجموعة ٢: الحيوانات المريضة بآلام البطن الحادة (٥٠)، انقسمت هذه الحيوانات مرة أخرى إلى ٣ مجموعات فرعية ، (١٠) من الخيول يعانون من آلام البطن مصاحبة بالإسهال ، (١٨) من الخيول يعانون من تخمة الأمعاء و(٢٢) من الخيول يعانون من المغص التقلصى. اظهرت النتائج أن جميع الخيول التى تعانى من آلام البطن الحادة كانت تعانى من زيادة فى عدد ضربات القلب ومعدل التنفس بالأضافة لزيادة وقت إعادة تعبئة الشعرية الدموية، بالأضافة لفرط حركة الأمعاء في الخيول التى تعاني من المغص التقلصى والإسهال. ومع ذلك، كان هناك بطء كبير في صوت الأمعاء قد يصل الى غياب كامل لهذه الحركة في الخيول التى تعاني من تخمة الأمعاء. تبين من خلال تحليل الدم وجود تغيرات طفيفة في صورة الدم مع زيادة غير معنوية في نسبة حجم الخلايا المضغوطة وأنخفاض غير معنوي للعدد الكلى لكرات الدم البيضاء فى غالبية الخيول المصابة بأنواع آلام البطن المختلفة الأسهال. اوضحت نتائج مؤشرات النزف عن وجود انخفاضا معنوياً في عدد الصفائح الدموية (PLTx10⁴/ μl) في الخيول التى تعانى من ألام البطن المصاحبة بالأسهال والخيول التى تعانى من التخمة (١٤.٣±١.٣٥ و ١٤.٩ ± ٠.٧٨) على التوالي) وزيادة معنوية في زمن البروثرومبين (/ثواني) فى الخيول التى تعاني من المغص التقلصى (١٤± ٠.٧٥) وزيادة غير معنوية فى باقى الفئات المرضية المصابة بالأسهال وتخمة الأمعاء (١٢.٤ ± ٠.٥٧ ،١٢.٧± ٣٧.١ على التوالي). كما تبين وجود زيادة معنوية فى زمن الثرومبوبلاستين الجزئي الفعال (/ثواني) في الخيول التى تعانى من المغص التقلصى (٥٣.٧٩ ± ٢.٤٤) وزيادة غير معنوية فى الفئات المرضية المصابة بالأسهال وتخمة الأمعاء (٤٨.٣ ± ٥.٧٥ ، ٤٥.٧ ± ٤.١٣ على التوالي). كما أن هناك زيادة غير معنوية في تركيزات الأنتى ثرومبين III (مليجرام/١٠٠مل) فى جميع الفئات المرضية المصابة بالأسهال ، تخمة الأمعاء والمغص التقلصى (٢٩ ± ٣.٤٢ ، ٢٩.٧±٣.١٩ ، ٢٩.٦± ٢.٧٣ على التوالى). أظهرت نتائج تحليل بروتين المرحلة الحادة وجود زيادة معنوية في مصل اميلويد (أ) (ميكروجرام/مل) في الخيول المصابة بالأسهال والمغص التقلصى (٢.٠٣٥ ± ٠.٥٠ ، ١.٦ ± ٠.١٦ على التوالى)، وزيادة غير معنوية فى الحيوانات التى تعانى من تخمة الأمعاء (١.٣٢± ٠.٠٨٩).

خلصت الدراسة إلى ظهور تغيرات فى مؤشرات النزف في الخيول التي تعاني من آلام البطن ولها تأثير معنوى على عدد الصفائح الدموية ، البروثرومبين ، الثرومبوبلاستين الجزئي الفعال والأنتى ثرومبين III . كما خلصت الدراسة الي ان مصل الأميلويد أ هو مؤشر قوى لآلام البطن في الخيول ، ويمكن استخدامه كعلامة بيولوجية تشخيصية لمغص الخيول.

الكلمات الكاشفة: خيول ، مغص ، مؤشرات النزف، مصل الأميلويد (أ)

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