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Altered of Serum Amyloid A, Haptoglobin, Coagulation Profile, and Venous Blood Gases of Arabian Horses Suffering from Spasmodic and Flatulent Colic in Egypt

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EQUINE colic is a common issue in the field of equine medicine, and it is considered as the number one cause of death among equines. The most common types of colic are spasmodic and flatulent colic. Dealing with a case of colic requires accurate decision and timing in which acute phase proteins (APPs), coagulation profile and venous blood gases can help to achieve that beside keen physical examination. This study includes thirty-two Arabian horses, in which 10 healthy horses used as control group, six were suffering from spasmodic colic, and sixteen were suffering from flatulent colic. All horses expressing colic signs are evaluated between 2-5 according to Equine Acute Abdominal Pain Scale (EAAPS). Cases were clinically evaluated based on vital signs, intestinal borborygmi, nasogastric intubation, and rectal examination. Jugular blood samples were collected and tested for complete blood count, coagulation profile, serum amyloid A (SAA), haptoglobin, and venous blood gas (VBG) analysis. The results showed a significant decrease in red blood cells and hemoglobin contents in flatulent colic cases, a significant increase in serum D-dimer, and SAA concentrations with a significant decrease in fibrinogen in both groups. While haptoglobin showed no alterations in both groups. Venous blood gases showed a significant decrease in pCO_2, HCO^-_3, and tCO_2 in both colic groups along with a significant decrease in ionized calcium (iCa^{2+}), and base excess with a significant increase in anion gap (AG) in the flatulent colic group compared to healthy group. Our conclusion indicates that SAA, serum D-dimer, and fibrinogen could be promising indicators of colic with no significant value in haptoglobin.

Keywords: Arabian Equine Colic, Serum Amyloid A, Haptoglobin, Coagulation profile, Venous blood gases.

Introduction

Horses' greatest health problem is equine colic, which is also the primary cause of morbidity and mortality as well as early deaths [1]. Flatulent colic, or gas in the intestinal lumen, and spasmodic colic are the two most common causes of colic [2,3]. When a horse has spasmodic colic, it experiences short bursts of intermittent abdominal discomfort during which it rolls, paws, and kicks for a few minutes before shaking itself and standing properly for a short while until the next episode of pain. It is common to hear intestinal sounds coming from a distance away from the horse, and auscultation will reveal loud, rumbling borborygmi. The pulse is increased moderately to around 60 beats per minute, and there may be some patchy sweating, but rectal examination is negative, and there is no diarrhea [4]. Undigestible feed can produce spasmodic colic and drastically change feeding patterns [5].

Abdominal distention was visible in flatulent colic patients, and there were severe signs of

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abdominal pain. Early on, peristaltic sounds are diminished; subsequently, they completely disappear. During a rectal examination, the abdominal cavity is filled with gas-filled loops of the large intestine, which prevents a thorough investigation of its contents [4].

Owing to the clinical and financial significance of colic, a precise diagnosis can be made through a combination of the patient's medical history, physical examination (PE) findings, nasogastric intubation (NGT), and rectal examination. Diagnostic tools that can be used to distinguish between different types of colic include imaging and clinicopathologic data [3].

Many studies have employed inflammatory markers to identify equine colic early on and determine the prognosis. It has been documented those horses suffering from colic exhibit abnormally elevated levels of Acute phase proteins (APPs) in their sera [6]. The administration of equine patients in both general practice and specialized hospital settings now requires the use of serum amyloid A (SAA) [7].

Haptoglobin's main job is to bind free hemoglobin and stop iron loss [8]. Haptoglobin has been examined recently during colic and is thought to be a moderate positive APP in horses. Its function in colic is still unknown, though. The body is shielded by hemoglobin from the damaging biochemical effects of myoglobin and free hemoglobin. Hemoglobin and myoglobin bound to haptoglobin stay confined inside the intravascular space, preventing injury to endothelial or renal tubular cells. The concentration of plasma haptoglobin drops quickly as a result of the liver removing haptoglobin-hemoglobin/myoglobin complexes [9].

It is unclear how hemostatic changes and fibrinolysis fit into the pathophysiology of colic in Arabian horses. A few published studies have used various coagulation and fibrinolysis abnormalities, including thrombocytopenia, prolonged clotting times (Prothrombin time (PT) and partial thromboplastin time (PTT)), decreased fibrinogen concentrations, increased d-dimer concentrations, and decreased antithrombin III (AT-III) activities, to predict gastrointestinal (GI) disorders in horses and diagnose subclinical disseminated intravascular coagulation (DIC). In horses with colic, the combination of prolonged PT, elevated PTT, and hypofibrinogenemia may be a good indicator of prognosis [10].

This study aimed to investigate the changes in hematobiochemical profiles, and venous blood gases together with the detection of coagulation profiles including PT, PTT, and D-Dimer. It also explores the predictive value of using inflammatory markers including SAA and Haptoglobin in early diagnosis of spasmodic and flatulent colic horses compared to healthy control horses.

Material and Methods

Animals

The questionnaire and methodology for this study were approved by the Institutional Animal Care and Use Committee of the Cairo University CU-IACUC (Ethics approval number: Vet CU 2009 2022502).

Thirty-two Arabian horses of both sexes (10 males and 22 females) aged from 1 year to 18 years of which 10 clinically healthy horses joined as the control and 6 horses with spasmodic colic and 16 horses with flatulent colic. The history, clinical symptoms, and physical findings were recorded. The type of feeding was forages (Egyptian clover, and Egyptian clover hay) introduced three times daily beside wheat straw which is the litter bedding of the stall, and fed ad lib, in addition to concentrates (barley and commercial pelleted feed) from 0.5kg to 2kg divided into 2-3 times over the day.

The severity of colic and pain scores were categorized according to [11]. Samples were collected from different private studs in Elreagha, Green Belt, Faculty of Veterinary Medicine Cairo University, Giza Governorate, Egypt, and Riding clubs in Madinat Nasr, Cairo governorate between October 2022 and October 2023.

Nasogastric intubation

Nasogastric intubation has been performed according to [12] using a nasogastric tube 2.7 m x 17 mm with funnel-shaped end (H. Hauptner and Richard Herberholz GmbH & Co. KG, Germany).

Samples

From the jugular vein of each horse, the blood sample was withdrawn and divided into three parts. The first one was collected on an EDTA tube for hematological examination using an automated veterinary hematology analyzer (IDEXX Lasercyte ®, USA). The second part was collected on a sodium citrate tube for plasma separation and coagulation test (PT, PTT, and fibrinogen concentration) was sent to a private lab. The third part was collected on a plain tube

for serum separation. The collected serum was stored at -20°C till use.

Serum samples were used to measure serum amyloid A (SAA), haptoglobin (Hpt), and D-dimer (D2D) concentrations by using equine-specific commercially available ELISA assay kits supplied by (Chongqing Biospes Co., China) according to manufacturer instructions. Serum amyloid A was measured according to [13], Equine haptoglobin was measured as in [14] and measurement of D-dimer as described in [15].

Another blood sample was taken from the jugular vein using a heparinized syringe with a 23G needle to measure venous blood gases (VBG) and electrolytes using (Sensacore ST-200 CC ABGE Blood Gas Analyzer, India) according to manufacturer instructions. As described in [16].

The obtained data were analyzed using the Independent-Samples T-test, the SPSS software package for Windows Ver. 20.0 (SPSS Inc., Chicago, IL, USA) and tabulated as mean value ± SE at levels of significance $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$. The p-value of $p \leq 0.001$ was considered highly statistically significant.

Results

Comprehensive clinical examination and physical findings associated with Arabian horses suffering from spasmodic and flatulent colic were tabulated in Table 1 and Fig 1. Also, colic pain scores were detailed according to the EAAPS (Equine acute abdominal pain scale) as described by [11] as shown in Table 2 and Fig 2

Laboratory findings

Our hematological results showed a significant decrease in both Hb content and RBC count ($p \leq 0.05$ and $p \leq 0.01$respectively) in the flatulent colic group with a non-significant decrease in the spasmodic colic group compared to the healthy control group (Table 3).

Regarding coagulation profile and APPs, results of fibrinogen concentrations, and D-Dimer were significantly decreased ($p \leq 0.01$) and increased ($p \leq 0.05$), with increases in SAA in both groups ($p \leq 0.001$ and $p \leq 0.01$ respectively) in both spasmodic colic and flatulent colic groups with no alterations in platelets, PT, PTT and haptoglobin concentrations compared to healthy group (Table 4).

Findings of venous blood gases showed significant decrease in pCO_2 ($p \leq 0.001$), HCO^-_3 ($p \leq 0.01$), and tCO_2 ($p \leq 0.001$, $p \leq 0.01$) in spasmodic and flatulent colic groups respectively along with significant decrease in ionized calcium ($p \leq 0.01$) and base excess ($p \leq 0.05$) with significant increase in anion gap ($p \leq 0.01$) in flatulent colic group compared to healthy group (Table 5).

Discussion

This study showed that most studied Arabian horses with spasmodic and flatulent colics are expressing abdominal pain between (2 - 5) on the EAAPS as described by [11]. Upon physical examination, most of the cases of both groups reveal a heart rate below 60 which is consistent with mild to moderate abdominal pain [17]. Respiratory rate was slightly increased in some cases and in others was normal, rectal temperature was normal in all cases of both groups except one case of the flatulent colic group. Intestinal borborygmi are mostly increased with a rumbling sound to normal in cases of spasmodic colic while in cases of flatulent colic intestinal borborygmi were mostly decreased [4]. Mucous membranes are normal in all cases of spasmodic colic and in most cases of flatulent colic only five cases were congested [18]. Our results agreed with the findings of [4] in spasmodic and flatulent colic cases. Most cases were evaluated as mild to moderate non-surgical as described in [18]. Rectal examination findings showed distention of the small intestine and colon in case of flatulent colic with no lesions and no abnormalities at all in case of spasmodic colic which came in accordance with [4].

Hematological parameters are mostly normal within the reference ranges in cases of survivor cases of colic as spasmodic and flatulent colic also reported by [10]. However, our study shows a significant decrease in RBC count and Hb concentration in the case of flatulent colic compared with the control group which may be due to some of the cases suffering from anemia.

While coagulation profile tests including platelets count, PT, PTT, fibrinogen and D-dimer concentrations showed no alterations in both spasmodic and flatulent colic groups except for serum fibrinogen and D-dimer concentrations which are significantly decreased and increased respectively in both groups which came in accordance with [10].

TABLE 1. Colic signs, severity, and physical examination findings in Arabian horses with spasmodic and flatulent colic.

Abdominal pain	Spasmodic colic (n=6)	Flatulent colic (n=16)
Restlessness	5	15
Curling upper lips up	0	5
Kicking at the belly	0	4
Looking at the belly	1	15
Paw at the ground	2	12
Laying down	5	16
Rolling	1	6
Colic Severity		
Mild	4	3
Moderate	1	7
Severe	1	6
Abdominal distension		
Absent	6	7
Present	0	9
Dehydration		
Absent	3	10
mild	3	1
Moderate	0	5
Severe	0	0
Intestinal motility (defecation)		
Normal	5	9
Absent	0	0
Constipation (scanty)	1	4
Diahrea (soft contains water)	0	3
Intestinal sound (borborygmi)		
Normomotility	4	0
Hypomotility	0	13
Hypermotility	2	2
Ileus	0	1
Appetite		
Off food	1	7
Poor (inappetent)	0	1
Good	5	8
Heart rate		
<44 beat/min	4	7
<60 beat/min	2	8
<80 beat/min	0	1
Profuse sweating	0	4
Urination		
Normal urination	6	12
Decreased urination	0	4
Frequent urination	0	0
Mucous membranes		
Normal	6	10
Congested	0	5
Petechial hemorrhage	0	1
Body temperature		
Normal	6	15
Elevated	0	1
Nasogastric intubation		
NA*	2	7
No reflux	4	4
Reflux	0	5
Rectal examination		
NA	3	11
Normal	3	0
Large intestinal tympany	0	5
Anatomical lesion	0	0

(*) NA = Not Available, not performed because of the nature of the case or safety concerns.

Fig. 1. A) Feces showing the presence of undigested barley grains obtained from 9-year-old Arabian mare suffered from spasmodic colic supposed to be secondary to sharp teeth. B) Feces obtained from the rectum during rectal examination covered by mucous because of decreased intestinal borborygmi obtained from 5-year-old Arabian mare suffered from flatulent colic. C) Huge reflux (mainly feed particles and straw) obtained from 12-year-old Arabian mare suffered from severe flatulent colic. D) Congested conjunctival mucous membrane obtained from 12-year-old Arabian horse suffered from severe flatulent colic.

TABLE 2. Colic pain score based on EAAPS* in Arabian horses suffering from spasmodic colic and flatulent colic.

Colic severity	Spasmodic colic (n=6)	Flatulent colic (n=16)
Score 0	0	0
Score 1	0	0
Score 2	4	3
Score 3	1	5
Score 4	0	2
Score 5	1	6

(*) EAAPS = Equine acute abdominal pain scale

Fig. 2. A) Arabian horse show Pawing (EAAPS 3) as a sign of spasmodic colic. B) Arabian mare showed sternal recumbency with attempting to lie down with flank staring (EAAPS 4) as a sign of severe flatulent colic. C) Arabian mare showed lateral recumbency (EAAPS 4) with severe abdominal distention as a sign of severe flatulent colic. D) Arabian mare suffered from severe flatulent colic showed rolling on the ground. (*) EAAPS = Equine acute abdominal pain scale.

TABLE 3. Hematological profile of spasmodic colic and flatulent colic Arabian horses compared to normal group.

Parameters	Control (n = 10) Mean± SE	Spasmodic colic (n=6) Mean± SE	Flatulent colic (n=16) Mean± SE
Hemoglobin (gm %)	13.53 ± 0.51	12.60 ± 0.76	11.44 ± 0.54^{c}
RBCs (10^6/mm^3)	7.78 ± 0.31	7.34 ± 0.61	6.36 ± 0.30^{b}
PCV %	34.33 ± 1.30	35.50 ± 1.91	34.00 ± 1.58
MCV (fl)	44.84 ± 0.54	45.10 ± 0.92	46.53 ± 1.15
MCH (pg)	17.31 ± 0.38	16.98 ± 0.52	17.46 ± 0.25
MCHC (g %)	37.48 ± 0.58	37.83 ± 0.71	37.61 ± 0.51
WBCs (10^3/mm^3)	9.37 ± 0.64	10.62 ± 1.16	8.65 ± 0.84
Neutrophils (10^3/mm^3)	6.37 ± 0.45	6.91 ± 0.81	6.00 ± 0.77
Lymphocytes (10^3/mm^3)	2.18 ± 0.51	2.69 ± 0.60	1.79 ± 0.19
Monocytes (10^3/mm^3)	0.56 ± 0.10	0.79 ± 0.25	0.59 ± 0.14
Eosinophil (10^3/mm^3)	0.25 ± 0.03	0.21 ± 0.02	0.25 ± 0.03

a: $p \leq 0.001$: highly significant; b:$p \leq 0.01$; c: $p \leq 0.05$; NS: Non-significant.

TABLE 4. Coagulation profile and acute phase proteins of spasmodic colic and flatulent colic groups compared to the control group.

Parameters	Control (n = 10) Mean± SE	Spasmodic colic (n=6) Mean± SE	Flatulent colic (n=16) Mean± SE
Coagulation profile			
Platelets (10^3/mm^3)	179.50 ± 0.17	171.00 ± 0.19	165.62 ± 0.07
PT (sec)	9.44 ± 0.23	10.00 ± 0.34	9.60 ± 0.41
aPTT (sec)	43.60 ± 2.92	49.83 ± 3.00	50.35 ± 2.61
Fibrinogen (mg/dL)	350.60 ± 0.22	227.33 ± 0.34[b]	219.14 ± 0.28[b]
D-Dimer (ng/mL)	561.47 ± 0.15	663.90± 0.37[c]	634.17 ± 0.25[c]
Acute Phase Proteins			
SAA(mg/dL)	43.79 ± 3.21	73.26 ± 3.19[a]	62.37 ± 4.58[b]
Hp (mg/dL)	70.66 ± 3.19	68.58 ± 3.45	68.11 ± 1.27

a: p ≤ 0.001: highly significant; b:p ≤ 0.01; c: p ≤0.05; NS: Non-significant.

TABLE 5. Venous blood gases in the spasmodic colic and flatulent colic group as compared to the control group.

Parameters	Control (n = 10) Mean± SE	Spasmodic colic (n=6) Mean± SE	Flatulent colic (n=16) Mean± SE
pH	7.37 ± 0.015	7.38 ± 0.02	7.39 ± 0.009
pCO_2(mmHg)	44.03 ± 0.41	34.84 ± 2.65[a]	34.71 ± 1.98[a]
Potassium (mmol/L)	3.93 ± 0.22	3.79 ± 0.36	3.47 ± 0.18
Sodium (mmol/L)	138.79 ± 0.73	138.54 ± 0.01	139.37 ± 1.27
Chloride (mmol/L)	95.74± 0.57	96.16 ± 0.82	95.73 ± 1.3
Ionized Calcium (mmol/L)	1.46 ± 0.02	1.43 ± 0.05	1.33 ± 0.02[b]
HCO^-_3 (mmol/L)	26.22 ± 0.29	23.52 ± 0.71[b]	22.61 ± 1.11[b]
tCO_2 (mmol/L)	27.54 ± 0.29	24.56 ± 0.72[a]	23.62 ± 1.15[b]
Base excess	1.42 ± 0.36	-.31 ± 0.93	-1.21 ± 1.15[c]
Anion gap (mmol/L)	20.75 ± 0.46	22.94 ± 1.73	24.49 ± 0.96[b]

a: p ≤ 0.001: highly significant; b:p ≤ 0.01; c: p ≤0.05; NS: Non-significant.

In relation to APPs, there was a significant increase in both groups' serum amyloid A concentration. In numerous circumstances, SAA rose; as [7] noted, in colic, it rose in reaction to pain. Studies have demonstrated that serum amyloid A (SAA) is a useful predictive too, since horses with increased SAA upon admission exhibit worse survival rates [19]. The most sensitive variable evaluated in relation to the need for surgical intervention or the emergence of complications in horses exhibiting symptoms of acute abdominal pain was SAA concentration, according to the current study's results [6].

However, neither group's blood haptoglobin concentration changed much, which is consistent with [9], who reported that haptoglobin is thought to be a moderately positive APP in horses and has recently been researched during colic. In horses, hemoglobin is regarded as a mild form of acute phase protein (APP); it starts to rise 12 to 24 hours after an inflammatory event and might be an appropriate indicator of a persistent inflammatory disorder [20]. Additionally, our research supports that of [6], which found that the three groups of horses in the current study did not significantly differ in their median serum haptoglobin concentration at hospital admission. This finding suggested that haptoglobin concentration was not a reliable diagnostic or prognostic indicator for horses exhibiting symptoms of acute abdominal

pain. Furthermore, the emergence of problems was not linked to an unusually elevated haptoglobin content. The rise in haptoglobin doesn't happen until 12 to 24 hours following an inflammatory stimulation.

Venous blood gas analysis shows a significant decrease in pCO_2, HCO^-_3, and tCO_2 in spasmodic and flatulent colic groups along with a significant decrease in ionized calcium (iCa^{2+}) and base excess with a significant increase in anion gap (AG) in flatulent colic group compared to healthy group. These findings. Showed agreement with [16] who found that, as compared to horses without symptoms of gastrointestinal illness, horses with colic had significantly higher anion gaps and significantly lower venous iCa^{2+}, HCO_3^-, tCO_2, and Base excess (BE). Unlike ours, these authors did not discover any variations in pCO_2. Similar to our work, [21] likewise discovered drops in venous HCO_3^- and Base excess (BE) concentrations between colic-ridden and healthy horses; however, they did not observe variations in pH, AG, or $PvCO_2$.

In a recent study, [22] arterial samples collected from the common carotid artery were used to compare the colic case data with the healthy horse case data. The colic horses showed reductions in all parameters, except for pH and anion gap, which were raised. Additionally, as reported by [22], $PaCO_2$ was lower in colicky horses. This was most likely a compensatory reaction to the metabolic acidosis brought on by lactate production and anaerobic metabolism, as evidenced by the lower values for HCO_3^- and Base excess (BE).

Sepsis, endotoxemia, and diarrhea can all result in a decrease in the serum Ca^{2+} content, as noted by [23]. Hypocalcemia was the most frequent electrolyte imbalance in colicky horses, as reported [16]. This outcome was also noted in prior research by [24], which found that during the preoperative timing, iCa^{2+} concentrations were lowered in horses suffering from colic and diarrhea as well as in surgical colic, as described by [23, 25]. Numerous factors, including endotoxemia, insufficient food intake, IV fluid therapy without calcium supplementation, ileus, sepsis, and variations in the percentage of calcium ionization as a result of blood pH and plasma albumin level changes, have been suggested as explanations for the hypocalcemia observed in colic horses [25].

Our investigation revealed large increases in the anion gap in the flatulent colic group. These increases might be the consequence of either an increase in unmeasured anions (lactate, phosphate, sulfate, ketoacids, albumin) or unmeasured cations (calcium, magnesium) as reported [26]. Alternatively, as described by [27], it might be the consequence of metabolic acidosis, as evidenced by the HCO_3^- concentration caused by hyperlactataemia.

Conclusion

From our clinical and physical findings together with laboratory words, we can conclude that serum D-Dimer and SAA can be used as prognostic indicators for survival in colic cases.

Authors' Contributions

All authors contributed to the study's conception and design. Data collection, clinical examination, and experimental study were performed by MHE, NEE, and MAE. All biochemical analysis and data analysis were performed by AHG and MHE. FAS, NEE, MHE, and AHG drafted and corrected the manuscript; NEE and FAS revised the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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The authors declare that the present study has no financial issues to disclose.

Conflict of interest

None

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التغيرات في Serum Amyloid A ، وال Haptoglobin، وملامح التخثر، وغازات الدم الوريدية في الخيول العربية التي تعاني من المغص التشنجي والمغص الانتفاخي في مصر

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يعتبر مغص الخيول من الحالات الأكثر شيوعا في مجال طب الخيل كما أنه يعتبر المسبب الأول لنفوق الخيل. يعتبر المغص التشنجي والمغص الانتفاخي أكثر أنواع مغص الخيل شيوعًا. التعامل مع حالات المغص يتطلب قرار وتوقيت جيد ويمكن أن يتحقق ذلك من خلال اختبارات البروتينات الحادة، التخثر وغازات الدم الوريدي بجانب الكشف والفحوصات الأساسية. شملت هذه الدراسة عدد اثنين وثلاثين حصان عربي بواقع عشر خيول يتمتعون ظاهريا بصحة جيدة تم استخدامهم كمجموعة تحكم وست خيول تعاني من المغص التشنجي بالإضافة إلى ستة عشر خيل يعانون من المغص الانتفاخي. جميع الخيول كانت تظهر أعراض المغص بدرجات بين ٢-٥ على مقياس ألم البطن للخيول EAAPS . جميعهم تم تقييمهم إكلينيكيا بناء على المؤشرات الحيوية، حركة الأمعاء، وضع الأنبوب الأنفي المعدي والفحص المستقيمي. تم تجميع عينات دم من الخيول محل الدراسة من الوريد الوداجي وذلك لاختبار صورة الدم الكاملة، اختبارات التخثر، قياس تركيز SAA و haptoglobin بالإضافة إلى تحليل غازات الدم الوريدي. أظهرت النتائج انخفاض بارز في عدد خلايا الدم الحمراء ونسبة الهيموجلوبين في حالات المغص الانتفاخي، ارتفاع بارز في تركيز D-dimer و SAA مع انخفاض بارز لتركيز الفيبرينوجين في كلا مجموعتي المغص. بينما لم يتأثر تركيز haptoglobin في كلا المجموعتين. أظهرت نتائج تحليل غازات الدم الوريدي انخفاض بارز في HCO_3^-، pCO_2 و tCO_2 في كلا المجموعتين مع انخفاض بارز في تركيز الكالسيوم المتأين (iCa^{2+}) وBE بالإضافة إلى ارتفاع بارز في AG في مجموعة المغص الانتفاخي بالمقارنة مع مجموعة التحكم.

استخلصنا أن قياس تركيز D-dimer ، SAA والفيبرينوجين من الممكن أن يكونوا مؤشرات واعدة لحالات مغص الخيول بالإضافة لعدم وجود قيمة تشخيصية لقياس تركيز haptoglobin في حالات المغص.

الكلمات المفتاحية: مغص الخيل العربية، Haptoglobin ،SAA، ملامح التخثر، غازات الدم الوريدية.