رقم البحث (1)

EVALUATION OF CLINICAL AND LABORATORY VARIABLES AS PROGNOSTIC INDICATORS OF GASTROINTESTINAL COLIC IN DRAFT HORSES

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ABSTRACT

A total of ninety native breed draft horses of both sexes were examined; from which, seventy five exhibited clinical signs of gastro-intestinal colic (forty seven were females and twenty eight were males) aged between one to eight years. In addition to fifteen apparently healthy horses of both sexes (ten females and five males) were randomly selected and served as a control group. Clinical parameters and laboratory variables were adopted to formulate prognosis in draft horses with gastro-intestinal colic. It is suggested that heart rate, respiratory rate; PCV%, total leucocytic counts; PCO2, PO2; bicarbonate gap, blood lactate and albumin concentrations are the most reliable determinants to predict the outcomes of gastro-intestinal colic in horses.

Key words: Clinical, Laboratory, Prognosis, Draft horses, Colic

INTRODUCTION

Colic considered as the most universally experienced health malady and was remained as the single most common cause of death so it became a continual concern for all horse owners. It was a frightening concept when a horse exhibited colic because it was often un-predictable and frequently not preventable (**Snyder and Spier, 1992 and Kaneene et al., 1997**). Colic was also an important cause of mortalities in domesticated horses and was

considered as the most frequently emergency conditions encountered in equine practices **(Pinsent and Fuller, 1997 and Reeves, 1997 and Zimmel, 2003).** Most previous studies were performed to identify clinical and laboratory variables that could be used to predict survival chances of the affected horses with colic prior to definitively determining its causes **(Ihler et al., 2004; Kahn et al., 2005 and Moore, 2006).**

Although a correct clinical diagnosis of the site and type of the intestinal lesion is often difficult, a correct diagnosis is necessary to predict a reliable prognosis **(Blikslager and Roberts, 1995).**

Clinical and laboratory variables such as heart rate, packed cell volume (PCV %), color of mucous membranes, capillary refill time (CRT), acid- base variables, anion gap and plasma lactate concentrations were valuable prognostic predictors **(Parry et al.,1983; Reeves et al., 1989; Pascoe et al., 1990; Sandholm et al., 1995, Furr et al., 1995 and Thoefner et al., 2000**). Based on the previously mentioned facts, the present study is planned to evaluate the usefulness of some clinical and laboratory parameters to formulate prognosis in horses with colic.

MATERIALS AND METHODS

1. Animals and Medical Records

A total of ninety native breed draft horses of both sexes were examined; from which seventy five cases exhibited clinical signs of gastro-intestinal colic (forty seven were females and twenty eight were males) aged between two to five years. In addition to fifteen apparently healthy horses of both sexes (ten were females and five were males) were randomly selected and served as a control group. Horses under investigation were fed on corn silage, rice hulls, rice polish, wheat bran and bean tibn in the dry season at a level of about 10 kg/head/day; however, berseem was offered in the winter one. This study was carried out between October, 2004 and october, 2008 at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt. Complete medical record for each animal was recorded depending on the competent history and clinical signs. Diagnosis of gastro intestinal colic was achieved by case history, physical examination, laboratory investigations and results of post-mortem examination.

2. Physical Examination: Rectal temperature, heart rate (bpm), respiratory rate (cpm), color of mucous membranes, capillary refill time, skin fold test, abdominal distension, peristaltic movement of alimentary tract, nasogastric reflux/L, results of rectal examination and severity of abdominal pain were recorded according to **Kelly (1984).**

3. A. Blood Samples: Two blood samples were collected from each animal; the first one was an arterial type whereas the second one was venous one.

3. A. 1. Arterial Blood Samples: Three ml heparenized syringe were used to obtain arterial blood from the transverse facial artery for estimation of blood pH, partial pressure of carbon dioxide (PCO2), partial pressure of oxygen (PO2), bicarbonate ($HCO3^-$), and base excess (BE) using blood gas analyzer *(AVL 995-Hb manufactured by AVL List GmbH Medizintechnik and distributed by AVL Medical Instruments UK Ltd)* according to **Reeves et al. (1989)** in addition to plasma electrolytes such as sodium and potassium which were measured using electrolyte analyzer (*AVL 984-S Analyzers manufactured by AVL List GmbH Medizintechnik and distributed by AVL Medical Instruments UK Ltd)* according to **Reeves et al. (1989)**. Chloride was also measured spectrophotometrically using commercial test kits supplied by (ABC diagnostics) according to the method described by **FeldKamp (1974).** The anion gap (AG) was calculated based on the equation AG = $(Na^+ + K^+)-(Cl^- + HCO3^-)$ adopted by **Feldman and Rosenberg (1981).** Bicarbonate gap was calculated as the difference between delta gap and delta bicarbonate where (delta gap = Calculated anion gap – 12, delta bicarbonate = 24 – measured bicarbonate) according to the method described by **Wrenn (1990) and Oster et al. (1988).** Blood glucose and lactate levels were also determined spectrophotometrically using commercial test kits supplied by (Diamond Company and Sigma Diagnostics, St Louis, Mo, USA) according to the method described by **Trinder (1969) and Noll (1974),** respectively.

3. A. 2. Venous Blood Samples

3. A. 2.i. Whole Blood: Two ml of blood were collected into a clean, dry vacutainer tubes with 5 mg sodium ethylene diamine tetra acetic acid (EDTA) as anticoagulant for hematological evaluation of packed cell volume (PCV %), total and differential leucocytic counts. Packed cell volume (PCV%) was determined using microhaematocrit tubes whereas total leucocytic count was carried out using hemocytometer and turkey's solution as diluents; however, differential leucocytic count was carried out using a stained blood film with Giemsa`s stain according to the method described by **Coles (1986).**

3. A.2.ii. Serum Samples: Ten ml of blood was obtained in a dry, clean and acid washed centrifuge tube without anticoagulant in order to obtain clear blood serum. Only clear, non-hemolysed sera samples were transferred into clean tubes and kept frozen until used for biochemical analysis of total serum protein, albumin, globulin, blood urea nitrogen and creatinine concentrations. The biochemical analysis of blood serum samples were determined spectrophotometrically using the commercial test kits supplied by (Boehringer Mannheim, ABC Diagnostics, Bio diagnostics and ABC Diagnostics) according to the method described by **King and Wootton. (1959), Drupt (1974), March et al. (1965) and Husdan and Rapoport (1968),**

3. B. Fecal Samples: Samples were collected directly from the rectum, and kept in plastic bags for parasitological examination using direct smear technique and floatation concentration technique for detection of internal parasites according to the method supplied by **Soulspy (1971).**

4. Post-Mortem examination: All non-survived cases were subjected to necropsy procedures which were performed according to the method described by **Tayler and Hillyer, (1997)**.

5. Statistical analysis: Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 15, USA) according to **Ihler et al. (2004).** Means and standard deviation for each variable was estimated. Differences between means in different types of colic were carried out using one way ANOVA with LSD post-hock multiple comparison test. Examination of the significance of different determinants to predict survival rate and site of colic was carried out using logistic regression. Firstly, univariate logistic regression was done, in which the state of horse was presented by dependent variable and all factors were presented by non-dependent variables. Factors significant at $P < 0.1$ were used for further stepwise multivariate logistic regression according to **Ihler et al. (2004).**

RESULTS

Table 1. Clinical diagnosis of horses under investigations

Groups	(n = 75)	%	Age	Gender
Spasmodic colic	22	29.3	2 – 4 years	Females (n = 15); Males (n = 7)
Gastric impaction	6	8.0	2- 8 years	Females (n = 2); Males (n = 4)
Large intestinal impaction	24	32	2 – 8 years	Females (n =16); Males (n = 8)
Flatulent colic	7	9.3	1- 4 years	Females (n = 4); Males (n = 3)
Small intestinal obstruction	6	8.0	2- 4years	Females (n = 4); Males (n = 2)
Large intestinal obstruction	5	6.7	5-8 years	Females (n = 3); Males (n = 2)
Verminous colic	5	6.7	1-4 years	Females (n = 3); Males (n = 2)

Table 2. Clinical and laboratory variables in survived and non- survived horses with different types of colic (means ± SD).

Variables	Animal status	
	Survived *(n = 54)*	**Non survived*(n = 21)***
Clinical findings		
▪ Heart rate	56.40 ± 1.28	82.52 ± 18.13
▪ Respiratory rate	17.75 ± 0.55	29.95 ± 1.86
Hematological findings		
▪ PCV%	38.29 ± 0.77	48.33 ± 1.17
▪ TLC $x10^3$	9.86 ± 0.31	6.88 ± 0.37
Blood gas results		
▪ pH	7.33 ± 0.006	7.22 ± 0.26
▪ PCO2	36.11 ± 0.55	35.43 ± 16.49
▪ PO2	66.16 ± 1.21	80.19 ± 3.31
▪ Bicarbonate	22.55 ± 0.47	20.28 ± 10.23
▪ Base excess	- 1.2 ± 0.54	- 4.09 ± 1.7
▪ Bicarbonate gap	0.70 ± 3.31	7.50 ± 3.31
Biochemical results		
▪ Sodium	131.38 ± 0.28	134.95 ± 0.21
▪ Potassium	3.5 ± 0.08	2.33 ± 0.66
▪ Chloride	98.25 ± 0.29	91.66 ± 1.32
▪ Anion gap	14.4 ± 0.41	20.5 ± 0.75
▪ Lactate	1.68 ± 0.07	4.05 ± 0.23
▪ Glucose	4.32 ± 0.45	6.96 ± 0.71
▪ Total serum protein	70.98 ± 8.93	85.66 ± 4.70
▪ Albumin	36.18 ± 7.71	46.61 ± 5.01
▪ BUN	5.36 ± 0.26	9.34 ± 0.34
▪ Creatinine	130.98 ± 2.06	154.38 ± 4.07

Table 3. Final multivariate stepwise logistic regression model for the predictive values in survived and non-survived draft horses with different types of colic.

Variables	P	OR	C.I at 95%
Clinical findings			
Heart rate	0.016	0.909	-
Respiratory rate	0.002	0.764	-
Hematological findings			
PCV%	0.001	1.496	1.168- 1.916
Total leucocytic counts	0.008	0.338	0.152- 0.750
Blood gases and acid base parameters			
PCo2	0.001	1.153	1.057- 1.258
PO2	0.029	0.923	0.859- 0.992
Bicarbonate gap	0.017	0.986	0.841-1.15
Biochemical investigations			
Lactate	0.004	48.750	3.488- 681.271
Albumin	0.038	1.356	1.017- 1.808

P: Significance values

OR: Odds ratio

C.I at 95%: Confidence interval

Significance of Different Determinants to Predict Survived and Non Survived Horses with Different Types of Colic

Clinical findings:

univariate analysis showed significant associations between non survived horses with increased heart rate ($p < 0.001$; OR: 1.143; CI at 95%: 1.076-1.215), respiratory rate ($p < 0.001$; OR: 1.422; CI at 95%: 1.210 1.671), abnormal mucous membrane color ($p < 0.001$; OR: 0.2; CI at 95%: 0.089- 0.448), rectal examination findings ($p < 0.004$; OR: 3.897; CI at 95%: 1.545-9.827), severity of clinical signs ($p < 0.001$; OR: 8.777; CI at 95%: 3.111-

24.764) and skin fold test (p < 0.003; OR: 1.981; CI at 95%: 1.069-3.668); However, multivariate stepwise logistic regression showed that there was significant association between non-survived horses with increased heart rate and respiratory rate (**Table 3).**

Hematological Findings:

univariate analysis showed that there was a significant association between non survived horses with increased PCV% (p < 0.001; OR: 1.27; CI at 95%: 0.278-0.651) and decreased total leucocytic counts (p < 0.01; OR: 0.425; CI at 95%: 0.278- 0.651)**;** However, multivariate stepwise logistic regression showed significant association between non-survived horses and increased PCV % with decreased total leucocytic counts at P < 0.001 and P < 0.008, respectively **(Table 3).**

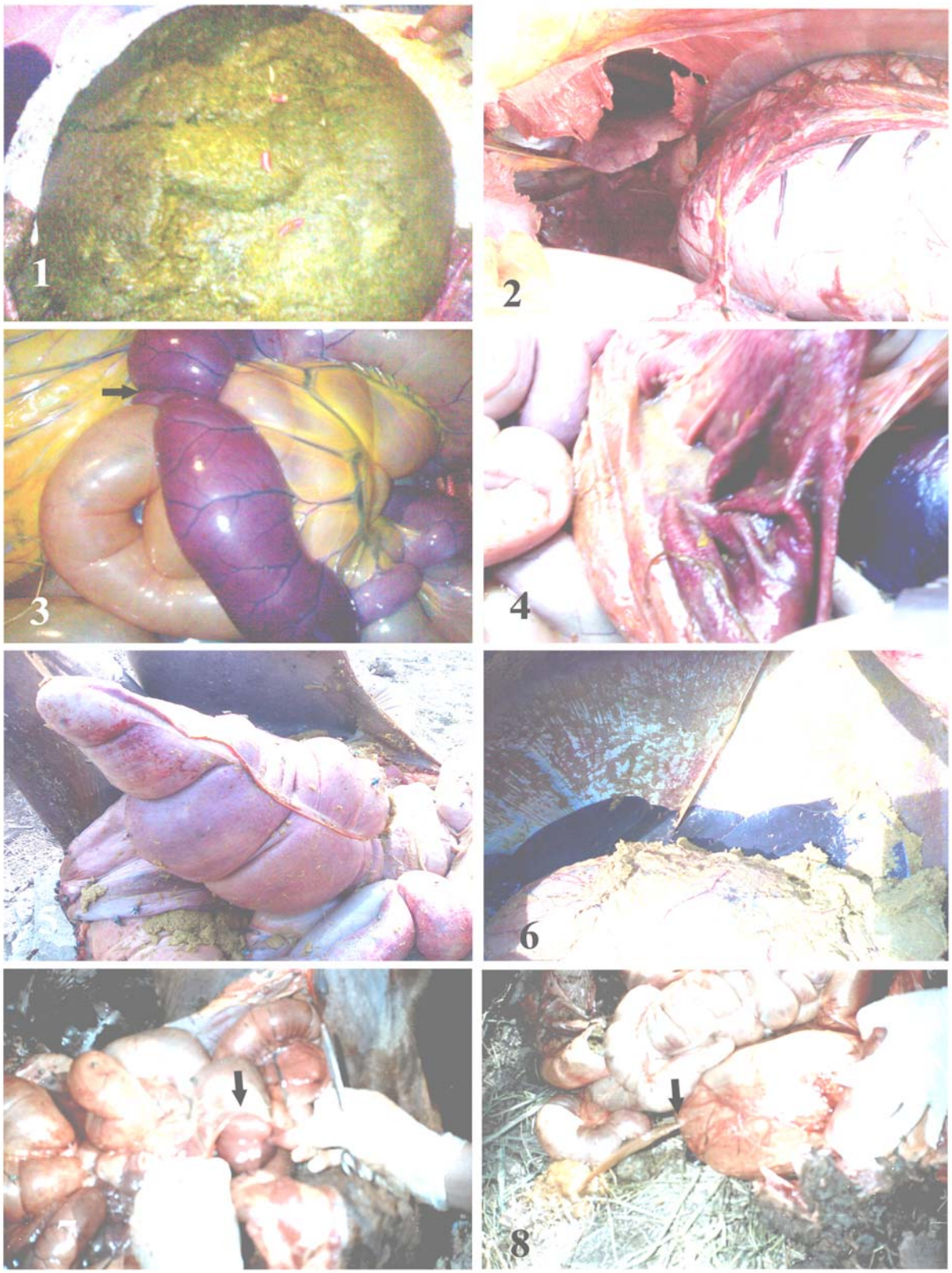
Blood Gases and Acid Base Parameters:

univariate analysis showed significant associations between non survived horses with decreased values of blood pH (p < 0.005; OR: 1.125; CI at 95%: 0.54), PCO2 (p < 0.002; OR: 1.140; CI at 95%: 1.049 - 1.238), PO2 (p < 0.05; OR: 0.937; CI at 95%: 0.877- 1.001), bicarbonate (p < 0.01; OR: 1.189; CI at 95%: 1.035- 1.366), base deficit (p < 0.044; OR: 1.125; CI at 95%: 1.003- 1.261) and increased bicarbonate gap levels (p < 0.001; OR: 1.189; CI at 95%: 1.269-2.117)**.** However, multivariate stepwise logistic regression showed significant association between non-survived horses and decreased values of PCO2, PO2 and increased values of bicarbonate gap **(Table 3).**

Biochemical Analysis:

univariate analysis showed significant associations between non-survived horses with colic and increased total serum protein (p < 0.001; OR: 1.589; CI at 95%: 1.267- 1.994), albumin (p < 0.001; OR: 1.350; CI at 95%: 1.179- 1.546), blood lactate (p < 0.001; OR: 25. 579; CI at 95%: 5.577- 117.3). plasma sodium (p < 0.001; OR: 2.897; CI at 95%: 1.754- 4. 784), glucose (p < 0.001; OR: 3.166; CI at 95%: 1.862- 5.385), anion gap (p < 0.001; OR: 1.866; CI at 95%: 1.383- 2.519), blood urea nitrogen (p < 0.001; OR: 1.589; CI at 95%: 1.267- 1.994) and creatinine (p < 0.001; OR: 1.082; CI at 95%: 1.042- 1.124) and decreased levels of potassium (p < 0.001; OR: 0.074; CI at 95%: 0.023- 0.243) and chloride (p < 0.001; OR: 0.535; CI at 95%: 0.379- 0.753). On the other hand, multivariate stepwise logistic regression showed significant association between non-survived horses and increased lactate and albumin concentrations **(Table 3).**

Fecal analysis revealed that large number of *Gastrophilus equi larvae* were detected in (4/6) of horses exhibited clinical signs of gastric impaction; however, *Strongylus* spp. eggs were detected in all cases (5/5) with clinical signs of verminous colic.



Necropsy findings:

All non-survived cases with obstructive colic were subjected to P. M examination where there was four non-survived cases because of cecal (Photo: 5) and pelvic flexure large colon impaction in addition to all cases with gastric impaction, small and large intestinal obstructive colic were also diagnosed by P. M examinations. At necropsy, there was severe distention of large colon at the pelvic flexure . Cyanoses and necroses of colon wall was also observed. Severe gastric dilatations caused by severe distention either by food as in cases of gastric impaction (Photo: 1) or by fluid as in cases of small intestinal obstruction (Photo: 8) were also detected. Large numbers of *Gastrophilus equi* larvae (Photo: 1) were also detected in (4/6) cases of gastric impaction. Congestion and cyanosis of small intestinal loops were also recorded in cases of small intestinal twisting (Photo: 3) and intussusception (Photo: 7). Two horses with gastric impaction showed rupture of diaphragm whereas one horse showed widespread soiling of internal organ with feed particle as a result of rupture of large colon because of severe impaction (Photo: 6).

DISCUSSION

Univariate analysis showed significant associations between non survived horses with increased heart rate ($p < 0.001$), respiratory rate ($p < 0.001$), abnormal mucous membrane color ($p < 0.001$), rectal examination findings ($p < 0.004$), severity of pain ($p < 0.001$) and skin fold test ($p < 0.003$). However, multivariate stepwise logistic regression showed significant association between non-survived horses with increased heart rate and respiratory rate **(Table 3).** It is suggested that, non-survived horses with colic could be predicted when heart rate and respiratory rate were 82.52 ± 18.13 (bpm) and 29.95 ± 1.86 (cpm), respectively. These findings were in agreement with those obtained by **Parry et al. (1983), Reinert (1986), Parry (1986) and Dabareiner and White (1995)** who reported that, there was significant difference ($P < 0.05$) for heart rate and respiratory rate between survived and non-survived horses with colic. The authors also added that, these variables were useful in predicting the chance of survival in horses with colic. Meanwhile, these findings were in disagreement with those reported by **Sandholm et al. (1995) and Thoefner et al. (2000)** who reported that heart rate, chloride concentration together with D-dimer were included in the final multi variable logistic regression. Unfortunately, fibrinogen could not be detected in all examined cases, so it was not tested for its significance to predict survival or non-survival in horses. However, **Ihler et al. (2004)** documented that heart rate and abnormalities concerning mucous membranes were the only statistically significant variables in the

multivariate model for surgical cases. Even if the initial laboratory variables didn't directly related to the outcome, these parameters were of importance in the decision making of supportive therapy such as fluid therapy and correction of acid-base disturbances. In this respect, they also might indirectly influence the outcome. However, in medical cases, PCV % was the only explanatory variable in the final logistic model. Also, PCV % represented a variable expressing the cardiovascular status in the patient. The increased heart rate and respiratory rate in non survived horses with different types of colic could be associated with poor peripheral perfusion and were considered as a reflection of cardiovascular collapse of the horse. This finding was in agreement with those reported by **Reinert (1986) and Thoefner et al. (2000).**

There was also significant correlation between the intensity of pain at time of admission ($p < 0.001$) and outcome of horses with colic. This finding was in agreement with those reported by **Sellers and Lowe (1986)** who mentioned that intensity of colic signs differed significantly between survivors and non- survivors. This index proved to have prognostic value. The authors also added that variations in the intensity of colic signs was greater in non-survivor group than in the survivor one but violence and lethargy were more common signs within the non-survivor group. On contrary, **Parry et al. (1983)** found that there was non- significant association between the severity of colic signs at admission and non-survival in horses with abdominal pain. This could be explained on the basis that most horses admitted to the referral centers were under the influence of analgesia so masking the real severity of colic signs.

The multivariate stepwise logistic regression showed that there was significant association between non-survived horses and increased PCV % ($p < 0.001$) and decreased total leucocytic counts ($p < 0.008$) **(Table 3).** It is suggested that non-survived horses with colic could be predicted when PCV% and total leucocytic were 48.33 ± 1.17 % and 6.88 ± 0.37 X 10^3, respectively (**Table 2).** The increased PCV % in non survived horses could be associated with development of hemoconcentration caused by dehydration with subsequent hypovolemic shock. Leucopenia in horses with colic however was frequently associated with intestinal mucosa damage with subsequent absorption of gram negative bacteria resulted in sepsis and endotoxemia. These results were coincided with those reported by **Parry et al. (1983), Pascoe et al (1990), Reinert (1986), Parry (1986) and Moore et al. (1986) and Radostits et al. (2007).**

Univariate analysis showed significant associations between non survived horses with decreased values of blood pH ($p < 0.005$), PCO2 ($p < 0.002$), PO2 ($p < 0.05$), blood bicarbonate ($p < 0.001$), base excess ($p < 0.044$) and increased values of bicarbonate gap ($p < 0.001$)**.** However, multivariate stepwise logistic regression showed significant association

between non-survived horses and decreased values of PCO2, PO2 and increased levels of bicarbonate gap **(Table 3)**. It is suggested that non-survived horses with colic could be predicted when the mean values of blood pH, PCO2 (mmol/L), PO2 (mmHg), blood bicarbonate (mmol/L), base excess (mmol/L) and bicarbonate gap (mmol/L) were 7.22 ± 0.26, 35.43 ± 16.49, 80.19 ± 3.31, 20.28 ± 10.23, - 4.09 ± 12.7 and 7.50 ± 3.31, respectively **(Table 2)**. Such findings could be attributed to the development of anaerobic metabolism associated with hypoxic state at the onset of shock (**Bristol, 1982).**

Univariate analysis showed significant associations between non-survived horses with increased total serum protein ($p < 0.001$), albumin ($p < 0.001$), and blood lactate ($p < 0.001$), plasma sodium ($p < 0.001$), glucose ($p < 0.001$), anion gap ($p < 0.001$), blood urea nitrogen ($p < 0.001$), creatinine ($p < 0.001$) and decreased chloride ($p < 0.001$) and potassium concentrations ($p < 0.001$)**.** Multivariate stepwise logistic regression showed significant association between non-survived horses and increased both blood lactate and serum albumin concentrations. It is suggested that non-survived horses with colic could be predicted when blood lactate and albumin concentrations were 4.05 ± 0.23 mmol/L and 47.2 ± 1.12 g/L, respectively **(Table 2)**. These findings were in harmony with those reported by **Donawick et al. (1975), Parry (1986) and Radostits et al. (2007)** who mentioned that, the probability of survival in horses with colic was decreased when the blood lactate was > 6.72 mmol/L. Moreover, the increase in blood lactate concentration could be associated with worsening in the peripheral perfusion at the onset of shock with eventually death. However, **Bristol (1982)** mentioned that the probability of survival decreased as the anion gap progressively increased above 20 mmol/L. Anion gap above 20 mmol/L was associated with 81 % survival; 20-24 mmol/L with 47 % survival; and >25% mmol/L with 0% survival. Meanwhile, the increased serum albumin concentration in non survivors could be explained by development of severe dehydration with subsequent hypovolemia and hemoconcentration. This finding was previously mentioned by **Dabareiner and White (1995), El-Ghareib (1997) and Robinson (2003)** who stated that up to 50 % of plasma volume might be lost after 18-24 hours in an acute bowel obstruction. In this study, P. M. examination had great clinical implications and depends on the underlying lesions, also it help confirm diagnosis of non-survived horses with gastrointestinal colic. This finding was in agreement with that reported by **Radostits et al. (2007).**

From the aforementioned results, it could be concluded that heart rate, respiratory rate; PCV%, total leucocytic counts; PCO2, PO2; bicarbonate gap, blood Lactate and albumin concentrations are the most reliable determinants to predict the outcomes of gastro-intestinal colic in horses.

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الملخص العربي

تقييم المتغيرات الإكلينيكية والمعملية كمؤشرات للتنبؤ

بالمغص المعدي المعوي في خيول الجر

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تم فحص عدد ٧٥ من خيول الجر المحلية تراوحت أعمارها بين سنة إلي ثماني سنوات منهم ٤٧ فرسا و٢٨ حصانا ظهرت عليهم جميعا علامات المغص المعدي المعوي. تم تقسيم هذه الحيوانات علي أساس تاريخ الحالة المرضي و الأعراض الإكلينيكية والتغيرات في الدم ومصله والصفة التشريحية بعد النفوق إلي ٢٢ من الخيول يعانون من المغص التقلصي (١٥ فرس و ٧ أحصنة) و ٦ خيول يعانون من لكمة المعدة (٢ فرس و ٤ أحصنة) و ٢٤ من الخيول يعانون من لكمة الأمعاء الغليظة (١٦ فرس و ٨ أحصنة) و٧ خيول تعاني من المغص النفاخي (٤ فرس و ٣ أحصنة) و ٦ خيول يعانون من التفاف الأمعاء الدقيقة (٤ فرس و ٢ حصان) و٥ خيول يعانون من التفاف الأمعاء الغليظة (٣ فرس و ٢ حصان) و٥ خيول يعانون من المغص الطفيلي (٣ فرس و ٢ أحصنة). وقد تم اختيار ١٥ من الخيول طبيعية من الناحية الظاهرية و المعملية كمجموعة ضابطة (١٠ أفراس و ٥ أحصنة). خلصت هذه الدراسة إلى أن هناك ارتباطا معنويا بين الخيول التي نفقت وزيادة كلا من معدل ضربات القلب و التنفس وزيادة حجم عمود الخلايا المضغوطة ونقص العدد الكلي لكرات الدم البيضاء وزيادة نسبة ثاني أكسيد الكربون ونقص الأوكسجين في الدم و زيادة مستويات الفارق في بكربونات الدم وزيادة معدل كلا من لاكتات وزلال الدم مما يوضح أهمية استخدام كلا من المتغيرات الإكلينيكية والمعملية كمؤشرات للتنبؤ بنتيجة المغص المعدي المعوي في خيول الجر.