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Research Article

Hematological and Biochemical Changes in Horses with Colic in Nairobi County, Kenya

Gitari AN*, Nguhiu-Mwangi J, Mogoa EM, Varma VJ, Mwangi WE, Konde AM and Rashid FK

Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya
***Corresponding author:** drgitari@gmail.com

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ABSTRACT

This was a prospective study, which involved following up cases of colic and from which blood samples for haematology and serum for biochemical analysis were collected. The haematological parameters measured were total erythrocyte count (TEC), haemoglobin concentration (Hb), Haematocrit (hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentartion (MCHC), red blood cell distribution width (RDW), platelet count, Total leucocyte count (TLC) and leucocyte differential count. The biochemical parameters measured were alkaline phosphatase (ALP), aspartate aminotransferase (AST), total proteins, serum albumin, serum globulin, blood glucose and serum lactate. The data obtained from the reported cases of colic was then imported into StatPlus pro 5.9.8 statistical package and means±SD were calculated and student t-test was then used to compare the means from horses that had impaction colic with those that had spasmodic colic as well as the means of those horses that recovered with those of the horses that died. The level of significance was at $P<0.05$.
Mean corpuscular haemoglobin (MCH) was significantly higher ($P=0.03$) in horses with spasmodic colic (16.8±1.3 pg) than in those with impaction colic (15.6±1.2 pg). The mean leucocyte count (10^9/L) was significantly higher ($p=0.02$) in horses with impaction colic (12.9±5.9) than in those with spasmodic colic (9.0±1.5). Similarly, mean neutrophil count (10^9/L) was significantly higher ($P=0.02$) in horses with impaction colic (9.1±5.6) than in those with spasmodic colic (5.4±1.7). The mean values of blood glucose were significantly higher ($P=0.02$) in horses with impaction colic (5.7±2.0 mmol/l) than in those with spasmodic colic (3.7±1.4 mmol/l). The blood biochemical parameters that were significantly lower in horses that died than those that survived were total protein ($P=0.002$), albumin ($P<0.01$) and globulin ($P=0.04$). Apart from Mean corpuscular hemoglobin (MCH), and slight leucocyte and neutrophil changes, there were no major significant haematological changes in horses with colic. Total blood proteins including albumin and globulin levels could serve as possible guides to prognosis of colic in horses. The general prognosis of spasmodic colic in horses was good, but fair to poor for impaction.

Key words: Horses, Colic, Hematology, Biochemistry, Diagnostic, Prognostic, Indicators

INTRODUCTION

Complete blood count (CBC) and biochemical profiles are commonly performed by equine veterinarians as the foundation of diagnostic evaluation with Packed Cell Volume (PCV), hematocrit, total protein; total leukocyte count, blood glucose and blood lactate being good prognostic indicators in horses suffering from acute abdominal disease. Packed Cell Volume (PCV) is an important prognostic indicator in horses with colic (Puotunen-Reinert, 1986). Elevated PCV has been shown to be a negative prognostic indicator for survival of the horse. However, other reports indicate that PCV has no prognostic significance (Van er Linden *et al*., 2003). It is therefore not wise to use PCV as the sole determinant of prognosis. Nevertheless, PCV is an indicator of cardiovascular compromise, which is an important determinant of survival (Parry *et al*., 1983). Low White Blood Cell Count (WBC) implies endotoxaemia and a probable indicator of devitalized intestine. Increased WBC is seen in impending colitis and peritonitis with signs of abdominal pain (Morris, 1991).

Total protein and albumin levels are important parameters in the management of horses with acute abdominal disease. A study showed that decreased serum total protein concentration at admission was associated

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with an increased risk of postoperative death in horses recovering from small intestinal surgery (Proudman *et al*., 2005). In another retrospective study of horses undergoing colic surgery, prognosis was found to be associated with total plasma protein, the type of lesion, preoperative PCV and the length of the surgical procedure (Pascoe *et al*., 1983). Hyperglycemia is common in horses with colic and is associated with poor prognosis, when considered together with changes in heart rate and PCV (Hassel *et al*., 2009). Horses with higher blood glucose concentrations have been associated with a less favorable prognosis at admission in hospital (Parry *et al*., 1983).

Plasma or peritoneal lactate levels are an important predictor for survival. In one study, blood lactate was found to be an important predictor of survival (Parry *et al*., 1983). Peritoneal lactate has been shown to be more useful predictor of intestinal ischemia secondary to strangulating obstruction than blood lactate (Furr *et al*., 1995). Plasma lactate concentration is elevated significantly in non-surviving horses with large colon volvulus and lower in horses with viable colon (Johnston *et al.*, 2007). Horses with increased blood or peritoneal lactate were reported to have a high likelihood of needing intestinal resection, developing postoperative ileus and increased probability of death (Delesalle *et al*., 2007).

Abdominal fluid analysis is also useful in determining if there is a need for surgery (Adams *et al*., 1980). Protein concentrations and complete white blood cell count of peritoneal fluid are useful in determining the degree of intestinal injury (Reeves *et al*., 1989). Increased protein concentration in abdominal fluid with no change in cell numbers is often due to simple obstruction with bowel distension. An increased number of white blood cells and ratio of neutrophils to monocytes (that is more than 70% neutrophils), will indicate a likelihood of bowel ischemia or degeneration with leakage of bacteria. Excess numbers of red blood cells (RBC) in the peritoneal fluid shows that there is leakage of cells from capillaries and this is an indicator for intestinal injury, particularly venous strangulation with obstruction. Increase of haemoglobin concentrations increases the odds that surgery is needed and increases the sensitivity and specificity of the decision compared to just visual assessment of peritoneal fluid. Increased peritoneal fluid lactate concentration compared to plasma lactate is also an indicator of intestinal compromise that indicates a surgical lesion. Increase of lactate concentration from a series of peritoneal fluid measurements is also an indicator of progressive intestinal injury (Delesalle *et al*., 2007). Bacteria in the peritoneal fluid are a clear indication of intestinal mucosal damage that allows their leakage through the intestinal wall (Weimann *et al*., 2002).

MATERIALS AND METHODS

Study area

This study was carried out in Nairobi County Kenya, which is located at 1°17′S 36°49′E, and has a daily temperature range between 16^0C and 30^0C. The horse population in the County is estimated to be 3,200 according to the Kenyan chapter of the international stud book committee. It was purposively decided to carry out the study in Nairobi County because of its relatively high population of horses, it is where the Jockey Club of Kenya is located, and it was convenient for the study. Nairobi County also has more equine practitioners compared to other Counties in Kenya. These factors made it possible to obtain the required sample size for the study.

Study design

The prospective study was carried out to assess the haematological and biochemical values in horses with colic. The haematological parameters evaluated were total erythrocyte count (TEC), haemoglobin concentration, haematocrit, mean corpusclar volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), and counts of platelets, Total leucocyte count (TLC), neutrophilis, lymphocytes, monocytes, eosinophilis and basophils. The biochemical parameters evaluated were alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins, serum albumin, random glucose and serum lactate. The study period for all the horses that had colic was November, 2015 to 31st march, 2016. The 27 horses that had manifested signs of colic and were reported had their blood samples taken from the jugular vein before any treatment was given. Diagnosis of colic (Appendix 1) was made by physical examination which included recognizing and assessing signs of abdominal pain, heart rate, respiratory rate and temperature. Other parameters determined included the colour of mucus membranes, capillary refill time and presence or absence of borborygmi sounds. Rectal examination and nasogastric intubation were done. Spasmodic colic was diagnosed based on findings of presence of increased borborygmi sounds (more than 3) in both fields of the abdomen, passing of gas and increased frequency of defaecation. Impaction colic was diagnosed based on findings of reduced borborygmi, bloated abdomen, presence of reflux through nasogatric intubation and rectal examination. Horses that did not show these signs of colic were excluded. A total of 10 ml of blood sample was collected from the jugular vein of each horse using direct venipuncture or a pre-placed IV catheter. Of this volume of blood, 4 ml was collected in vacutainer tubes with EDTA and 4 ml was collected in vacutainer tubes with clot activator and 2 ml in sodium fluoride tubes. The 4 ml of blood sample with EDTA for each horse with colic was used to analyze for total erythrocyte count (TEC) in 10^{12}/L, total platelet count (TPC) in 10^9/L, total leucocytes count (TLC) in 10^9/L, packed cell volume (PCV) in %, hemoglobin concentration (Hb) in g/dl and differential cell count (Neutrophils, Basophils, Eosinophils, Monocytes and Lymphocytes). The other 4 ml blood was assayed for alkaline phosphatase (IU/L), aspartate aminotransferase (AST) (IU/L), total protein (g/l) and serum lactate (mmol/l). The remaining 2 ml was assayed for random glucose (mmol/l). The hematological analysis was done using an automatic cell counter (Idexx Procyte DX Haematology Analyzer, Idexx laboratory, USA) following the manufacturer's instructions. The blood samples with the clot activator and sodium fluoride were immediately centrifuged at 3500 revolutions per minute for 10 minutes and serum was harvested. Serum samples were stored at -20ºC and analyzed using dry

chemistry technology with Idexx catalyst DX chemistry analyzer (Idexx laboratory,USA) for alkaline phosphatase (ALP), aspartate aminotransferase (AST), total protein, serum albumin, random glucose and serum lactate.

Relevant treatment was instituted for colic and the horses closely monitored until either recovery or death. The cases were categorized as either impaction colic or spasmodic colic from their clinical presentation and physical examination.

Initially, all the haemotological and biochemical data were first categorized into those from impaction colic horses or spasmodic colic horses as well as from horses that recovered or those that died. The haematological and biochemical data were entered into Microsoft Office Excel 2010 then verified and validated to be correct entries as per the data collection sheets. This data was then imported into StatPlus pro 5.9.8 statistical package where normality tests were first computed using Shapiro Wilk normality test. Following this, means±SD were calculated and student t-test was then used to compare the means between the categories of colic as well as between those that recovered and those that died at $P<0.05$ significance level.

RESULTS

Descriptive analysis

A total of 27 horses manifesting signs of colic were examined in this phase of the study. The cases were presented between November, 2015 and March, 2016. The horses were adults from 9 stables comprising of 15 females and 12 males. During this period, only one horse had recurring episodes of colic. There were 18.5% (n = 5) deaths. The findings from the history and physical examination of the reported cases pointed to spasmodic, impaction colic and torsion. Spasmodic colic was diagnosed in 55.6% (n=15) of the cases while impaction colic was in 40.7% (n=11) of the cases and that of torsion at 3.7% (n=1). The case of torsion was excluded from the analysis to restrict comparison to between impaction and spasmodic colic only. Postmortem of the 5 horses that died revealed torsion (1) at the jejunum and impaction at the transverse colon (2) and pelvic flexure (2) as the cause of death.

Mean values of hematological parameters in spasmodic and impaction colic

Haematological values of horses with spasmodic colic were compared with those of horses with impaction colic. The mean haematological values between horses with spasmodic colic and those with impaction colic were very similar, hence the differences were largely not significant ($P>0.05$) (Table 1), except for the mean corpuscular haemoglobin (MCH), total leucocyte count and neutrophil count. The mean corpuscular haemoglobin was significantly higher ($p = 0.03$) in horses with spasmodic colic [16.8±1.3 pg] than in those with impaction colic [15.6±1.2 pg]. The mean leucocyte count was significantly higher ($p = 0.02$) in horses with impaction colic [12.9±5.9 (10^9/L)] than in those with spasmodic colic [9.0±1.5 (10^9/L)]. Similarly, neutrophil count was significantly higher ($p = 0.02$) in horses with impaction colic [9.1±5.6 (10^9/L)] than those with spasmodic colic [5.4±1.7 (10^9/L)].

Mean values of haematological parameters in horses that recovered and those that died of colic

The mean values of the haematological parameters of the horses that recovered from colic and of those that died of colic were closely related with no significant differences ($P>0.05$) between them as shown in Table 2. Although the differences were not significant, overall, the mean values of total leucocytes were higher in the horses that died of colic than those that recovered. Similarly, the haematocrit and platelet mean values appeared to be much higher in the horses that died of colic than in those that survived, despite this difference being not significant.

Mean values of biochemical parameters in horses with colic

The mean values of blood glucose were significantly higher ($P=0.02$) in horses with impaction colic (5.7±2.0 mmol/l) than in those with spasmodic colic (3.7±1.4 mmol/l). There were no significant differences in the mean values of Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Total protein, Albumin and Serum lactate between the horses that had impaction colic and those that had spasmodic colic (Table 3). Despite there being no significant difference, the mean values of Alkaline phophatase and Aspartate aminotransferase were relatively higher in horses with impaction colic than in those with spasmodic colic. In addition, mean total proteins were relatively lower in impaction colic than in spasmodic colic cases.

Mean values of biochemical parameters in horses that recovered or died from colic

There were significant differences in the mean values of total protein, albumin and globulin between colicky horses that died and those that survived. The mean values of total protein were significantly lower ($P<0.01$) in horses that died (57± 5.4 g/l) than in those that survived (72.5±14.8). Similarly, the mean values of albumin were significantly lower ($P<0.01$) in horses that died (24.7±2.1 g/l) than in those that survived (33.7±7.6 g/l). The mean values of globulin were equally significantly lower ($P=0.04$) in horses that died (32.4±3.9 g/l) than in those that survived (38.7±9.1 g/l) the colic. On the other hand, and although not significant, alkaline phosphatase was relatively higher in horses that died than in those that survived from colic, while aspartate aminotransferase was relatively higher in those that survived than in those that died (Table 4).

DISCUSSION

In this prospective study there was higher total leucocyte and neutrophil counts in impaction colic, as well as in the horses that died as compared to those that recovered and this can be attributed to the fact that impaction applies intense pressure from the lumen to the walls of the involved segment of the gastrointestinal tract. When impaction persists over time, the pressure may result in slight degree of ischaemia, devitalization and degeneration of walls of the intestine. Subsequently, some bacteria die and release endotoxins into circulation and bacteria leak into blood, thus triggering a rise in neutrophils and total leucocyte count as has been suggested

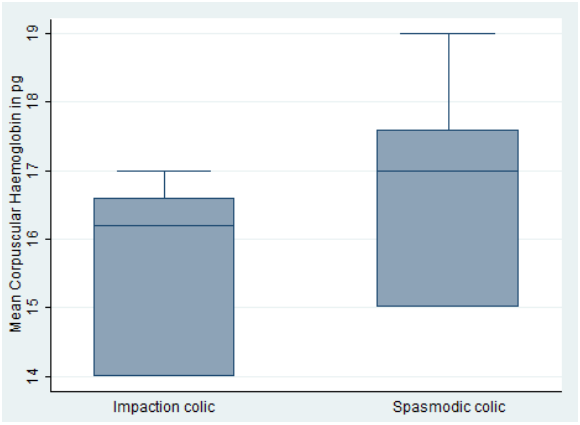

Fig. 1: A Box-and Whiskers plot comparing the means of the mean corpuscular haemoglobin (pg) between horses with impaction colic and spasmodic colic, in which the difference was significant (p=0.03).

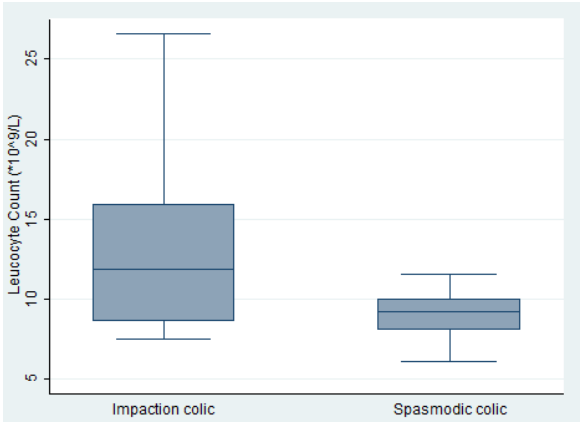

Fig. 2: A Box-and Whiskers plot comparing the means of leucocyte counts (10^9/L) between horses with impaction and spasmodic colic, in which the difference was significant (p=0.02).

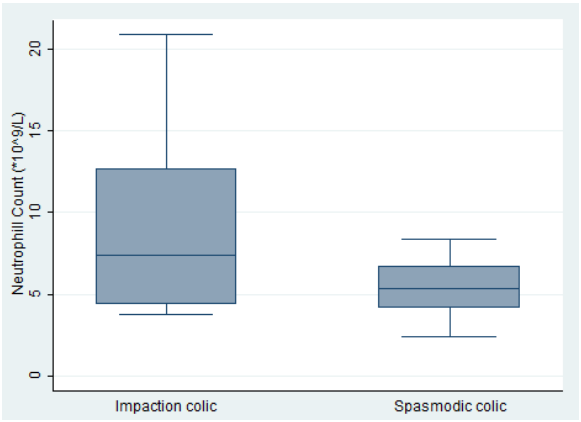

Fig. 3: A Box-and Whiskers plot comparing the means of neutrophil counts (10^9/L) between horses with impaction and spasmodic colic, in which the difference was significant (p=0.02).

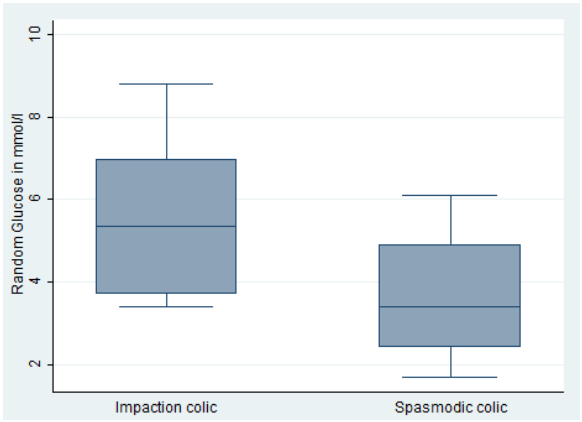

Fig. 4: A Box-and Whiskers plot comparing the means of random glucose (mmol/l) between horses with impaction and spasmodic colic, in which the difference was significant (p=0.02).

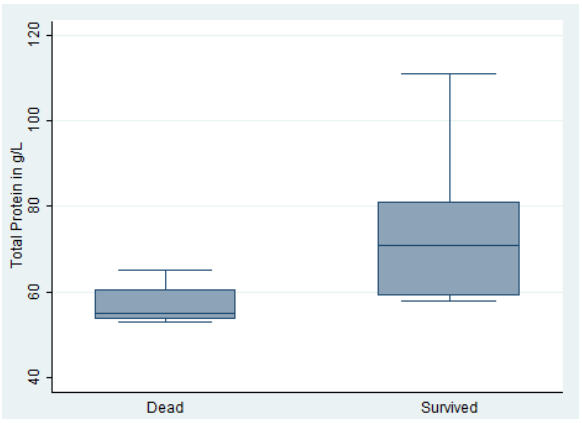

Fig. 5: A Box-and Whiskers plot comparing the means of total protein (g/l) between horses with impaction and spasmodic colic, in which the difference was significant (p=0.002).

previously (Morris, 1991; Weimann *et al.*, 2002). This may be the same reason for the death of some of the impaction cases of colic while those with spasmodic colic recovered.

Alkaline phosphatase (ALP) which is an enzyme present in intestinal mucosa although not significant was increased in horses with impaction than those with spasmodic colic and this was similar to a study that indicated an increase in serum ALP is associated with greater intestinal damage which leads to a likelihood of probability for surgery and a worse prognosis (Saulez *et al*., 2004).

Muscle enzyme Aspartate aminotransferase (AST) although not significant was relatively high in horses with impaction colic than those with spasmodic colic. This is similar to a study that found increase in pre-operative AST concentration were significantly associated with lesions associated with intestinal ischaemia and subsequent decrease of survival probability in horses in hospitals. Preoperative increase in AST enzyme activities will assist in finding out the severity and prognosis of horses with colic and make an informed decision if they should be going through celiotomy (Krueger *et al*., 2014).

The significantly higher mean values of blood glucose in horses with impaction when compared to those with spasmodic colic is similar to the previous findings in other studies that have reported hyperglycaemia in cases of colic even though in those studies, the types of colic were not designated (Hassel *et al.*, 2009). The reason for the hyperglycaemia is not well known, but it is suggested that the horses with colic develop temporary insulin resistance that leads to blood glucose elevation. It was

Table 1: Comparative mean haematological values between horses with impaction colic and those with spasmodic colic in Nairobi county between November 2015 and March 2016.

Haematological parameters	Type of colic	Means±SD	P Value
Erythrocyte count (10^{12}/L)	Impaction	9.6±1.4	0.20
	Spasmodic	8.9±2.1	
Hemoglobin concentration (g/dl)	Impaction	14.9±1.8	0.95
	Spasmodic	18.4±3.2	
Hematocrit (L/L)	Impaction	0.42±0.07	0.98
	Spasmodic	0.42±0.1	
Mean corpuscular volume (fl)	Impaction	43.2±5.6	0.13
	Spasmodic	46.3±2.8	
Mean corpuscular hemoglobin (pg)	Impaction	15.6±1.2	0.03*
	Spasmodic	16.8±1.3	
Mean corpuscular hemoglobin concentration (g/dl)	Impaction	36.3±2.8	0.81
	Spasmodic	36.0±2.3	
Red blood cell width (%)	Impaction	26.4±9.9	0.15
	Spasmodic	20.9±8.0	
Total platelet count (10^9/L)	Impaction	162.5±196.1	1.0
	Spasmodic	160.1±130.5	
Lymphocyte count (10^9/L)	Impaction	3.3±1.3	0.37
	Spasmodic	2.8±1.3	
Monocyte count (10^9/L)	Impaction	0.6±0.8	0.89
	Spasmodic	0.6±0.4	
Eosinophils count (10^9/L)	Impaction	0.20±0.1	0.17
	Spasmodic	0.2±0.2	
Basophil count (10^9/L)	Impaction	0.1±0.04	0.27
	Spasmodic	0.1±0.04	
Total leucocyte counts (10^9/L)	Impaction	12.9±5.9	0.02*
	Spasmodic	9.0±1.5	
Neutrophil count (10^9/L)	Impaction	9.1±5.6	0.02*
	Spasmodic	5.4±1.7	

*****Significant at P<0.05.

Table 2: Comparative mean values of haematological parameters between horses that recovered from colic and those that died of colic in Nairobi County from Novermber 2015 to March 2016

Hematological parameters	Outcome	Means±SD	P Value
Erythrocyte count (10^{12}/L)	Died	9.1±3.1	1.0
	Recovered	9.1±1.8	
Hemoglobin concentration (g/dl)	Died	14.3±2.2	0.7
	Recovered	14.9±2.7	
Hematocrit (L/L)	Died	11.3±21.9	0.39
	Recovered	0.4±0.1	
Mean corpuscular volume (fl)	Died	40.6±5.3	0.14
	Recovered	45.7±4.0	
Mean corpuscular hemoglobin (pg)	Died	17.1±5.2	0.8
	Recovered	16.4±1.3	
Mean corpuscular hemoglobin concentration (g/dl)	Died	41.5±7.2	0.22
	Recovered	36.0±2.4	
Red blood cell width (%)	Died	29.6±11.4	0.35
	Recovered	23.3±9.0	
Total platelet count (10^9/L)	Died	417.3±326.8	0.22
	Recovered	139.4±109.0	
Lymphocyte count (10^9/L)	Died	2.3±1.8	0.5
	Recovered	3.0±1.3	
Monocyte count (10^9/L)	Died	0.4±0.2	0.14
	Recovered	0.6±0.6	
Eosinophils count (10^9/L)	Died	0.12±0.2	0.86
	Recovered	0.14±0.2	
Basophil count (10^9/L)	Died	1.1±2.2	0.40
	Recovered	0.1±0.04	
Total leucocyte counts (10^9/L)	Died	18.1±7.2	0.13
	Recovered	10.4±4.4	
Neutrophil count (10^9/L)	Died	14.2±9.3	0.12
	Recovered	6.7±4.9	

Significant at P<0.05.

Table 3: Comparative mean values of biochemical parameters between horses with impaction colic and those with spasmodic colic in Nairobi County from November 2015 to March 2016

Biochemical parameters	Type of colic	Means±SD	P Value
Alkaline phosphatase (ALP) IU/L	Impaction	89.0±69.8	0.2
	Spasmodic	55.0±31.1	
Aspartate amino-transeferase (AST) IU/L	Impaction	453.4±225.3	0.9
	Spasmodic	436.5±222.6	
Total protein g/l	Impaction	67.9±17.8	0.45
	Spasmodic	73.0±13.3	
Albumin g/l	Impaction	30.4±7.2	0.19
	Spasmodic	34.5±8.1	
Globulin g/l	Impaction	37.5±7.2	0.8
	Spasmodic	38.5±8.1	
Blood glucose mmol/l	Impaction	5.7±2.0	0.02*
	Spasmodic	3.7±1.4	
Blood lactate mmol/l	Impaction	7.4±2.9	0.12
	Spasmodic	5.7±1.6	

* Significant at P<0.05.

Table 4: Comparative mean values of biochemical parameters between the horses that died and those that recovered from colic in Nairobi County from November 2015 to March 2016

Biochemical parameters	Outcome	Means±SD	P Value
Alkaline phosphatase (ALP) IU/L	Dead	120±64.9	0.19
	Survived	66.4±49.4	
Aspartate amino-transeferase (AST) IU/L	Dead	413±10.3	0.61
	Survived	439.7±225.4	
Total protein g/l	Dead	57±5.4	0.002
	Survived	72.5±14.8	*
Albumin g/l	Dead	24.7±2.1	0.000
	Survived	33.7±7.6	1*
Globulin g/l	Dead	32.4±3.9	0.04*
	Survived	38.7±9.1	
Blood glucose mmol/l	Dead	6.9±2.2	0.08
	Survived	4.2±1.7	
Blood lactate mmol/l	Dead	6.3±3.3	0.98
	Survived	6.2±2.3	

*Significant at P<0.05

further suggested that the cases of colic with hyperglycemia have worse prognosis for survival (Hassel *et al.*, 2009). A study related hyperglycemia in critically ill patients due to a consequence of deregulation of glucose homeostasis manifested by peripheral insulin resistance, hyperinsulinemia, increased gluconeogenesis and impaired peripheral insulin-mediated glucose uptake (Vanhorebeek *et al.*, 2007). Study of 269 horses with colic presented to a referral hospital within the United Kingdom showed about 50% of horses that had blood glucose concentrations higher than the normal range of 3.5-7.4mmol/l and higher glucose concentrations at the time of admission were associated with non survival (Hollis *et al.*, 2007). Although the blood lactate in impaction colic was not significantly different from spasmodic colic, it was nevertheless higher. This has been associated with poorer prognosis for survival in cases of colic with lactate at greater than 8 mmol/l (Moore *et al.*, 1976). Studies have indicated that several blood lactate analysis are more useful prognostic indicator than just single lactate analysis. Hospitalized horses with increased blood lactate concentration from serial measurements were seen in non

survivors compared to survivors, however this difference tend to be small (Tennent-Brown, 2012).

The significantly lower serum levels of total protein, albumin and glubulin in horses that died compared to those that recovered agrees with reports of a retrospective study, which cited total plasma protein as one of the factors that influenced survival in horses that underwent surgery for treatment of colic (Pascoe *et al.*, 1983). Similarly, in another study, it was stated that decreased serum total protein concentration was associated with an increased risk of postoperative death in horses recovering from small intestinal surgery. The decrease in plasma protein is an indication of loss of this protein through damaged intestinal walls (Proudman *et al.*, 2005).

Conclusion

It can be concluded from this study that the only significant haematological changes observed were slight elevation of leucocyte and neutrophil counts in impaction colic compared to spasmodic colic. Similar elevation of these parameters were also present in horses that died of colic compared to those that recovered from colic. Blood glucose was higher in horses with impaction colic than in those with spasmodic colic and this could be a useful prognostic factor to consider when managing impaction colic. Another factor that could be useful to consider in determining prognosis for survival particularly in cases with cases that may have devitalized intestine is total plasma or serum proteins. It is suggested that probably abdominal fluid analysis could be useful in diagnosis and prognosis of colic cases as suggested previously (Adams *et al*., 1980; Reeves *et al*., 1989; Weimann *et al*., 2002).

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