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Paper

Sensitivity and specificity of blood leukocyte counts as an indicator of mortality in horses after colic surgery

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The objectives of this study were to describe and relate perioperative changes in blood leukocyte counts to the outcome of surgical colic horses, determine a cut-off value in the early postoperative period to obtain an indicator of the outcome, and compare the obtained value to a validation population of horses. Fifty-three horses undergoing colic surgery were included in the descriptive part of the study. Total leukocyte counts were performed before, during and serially after surgery. A receiver operating characteristic analysis was performed on the leukocyte counts of 45 of these horses to determine a cut-off value for the outcome. The results obtained were validated on a second set of 50 horses that underwent colic surgery in similar conditions. The kinetics of blood leukocytes in survivors was higher than in non-survivors during the first days. Non-survivor horses were more likely to have at least one blood leukocyte count ≤3.9×10³/mm³ between 28 and 60 hours after surgery than survivor horses. This cut-off value was confirmed in the validation population. These results suggest that routine values of blood leukocyte counts can be used as an additional prognostic indicator after colic surgery alongside other predictors previously associated with the outcome.

Introduction

Despite the recent advances in the area of abdominal surgery, anaesthesia and postsurgical care, colic remains a major cause of death in horses. Different studies on equine mortalities conducted in a referral hospital (Baker and Ellis 1981), from insurance data (Leblond and others 2000) or conducted on several different horse populations (Wallin and others 2000) identified colic as cause of death in 5–33 per cent of the cases. Another study reported a mortality rate of 25 per cent for all types of colic admitted in a referral hospital (Sutton and others 2009). In surgical colic cases, survival rates ranging from 60 per cent to 70 per cent have been reported by referral centres (Abutarbush and others 2005, Mair and Smith 2005a, Krista and Kuebelbeck 2009, Sutton and others 2009).

During the past decades, numerous studies evaluated signalment, history, physical examination, clinicopathological data (including the evaluation of coagulopathies), lesion type and postoperative complications as prognostic indicators for survival/mortality and/or to distinguish medical verse surgical colic cases (Fischer 1989, Dukti and White 2009).

For surgical colic cases that recovered from anaesthesia, Mair and Smith (2005a) and Krista and Kuebelbeck (2009) described a short-term survival rate (to discharge) of 83.1 per cent and 88.3 per cent, respectively. Persistent pain, postoperative ileus, peritonitis, shock, endotoxaemia, colitis/diarrhoea, rupture of a portion of the gastrointestinal tract and laminitis were the most commonly reported causes of death or euthanasia after colic surgery (Proudman and others 2002, Mair and Smith 2005a, Krista and Kuebelbeck 2009, Prange and others 2010).

Leukocyte inflammation is related to many of these conditions: ileus (Little and others 2005), peritonitis (Dyson 1983, Feige and others 1997), development of inflammation/sepsis and endotoxaemia (Corley and others 2005, Epstein and others 2011), diarrhoea and/or salmonella shedding (Morris and others 1983, Dallap Schaer and others 2012) and laminitis (Faleiros and others 2011, Visser and Pollitt 2011).

A decreased blood neutrophil deformability was observed in colic horses and was related to the severity of the illness (Seahorn and others 1994). In strangulating colic, activated neutrophils were considered as a negative prognostic indicator (Weiss and Evanson 2003). However, blood leukocyte or neutrophil counts did not show any prognostic significance during initial examination at admission to the clinic (Seahorn and others 1994, van der Linden and others 2003).

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As serial blood leukocytes counts (total white blood cell counts) are routinely performed in our clinic in horses after colic surgery, we hypothesised that kinetics of the blood leukocytes counts were different between horses that survive to discharge and those that did not, and that a cut-off value determined in the early postoperative period may have a better prognostic value than at the time of admission.

Therefore, the aims of this study were (1) to describe the kinetics of blood leukocytes (total white blood cell counts) in the perioperative period of colic horses and to relate the evolution of the leukocytes to the outcome, (2) to determine a cut-off value in the early postoperative period as an indicator of the outcome of colic horses and (3) to compare the obtained cut-off value to 'prospective' cases.

Materials and methods

Horses

Fifty-three horses undergoing colic surgery at the Equine Clinic of the Faculty of Veterinary Medicine, University of Liège, Belgium were included in this study. These horses had a survival time of at least 12 hours postsurgery and were considered as 'population 1' for the descriptive part of the study. Horses euthanased due to financial reasons were excluded.

Signalment and clinical data were obtained from the medical records. All horses underwent a complete physical and rectal examination as well as a nasogastric intubation on admission. Venous blood gas analysis, haematologic and biochemical profiles were performed. Peritoneal fluid was recovered when available. All horses were treated surgically by ventral midline celiotomy under general anaesthesia. Anaesthesia was induced with ketamine 2.2 mg/kg and midazolam 0.06 mg/kg five minutes after premedication with 0.6 mg/kg of xylazine, all given intravenously. Orotracheal intubation was performed and anaesthesia maintained by isoflurane in oxygen or oxygen/air mixture. Intermittent positive pressure ventilation was installed in order to maintain normocarbia. Intra-anaesthetic parameters consisting of heart rate, respiratory rate, peak inspiratory pressure, tidal volume, end-tidal CO_2 pressure, end-tidal isoflurane concentration, oxygen saturation of haemoglobin, invasive mean arterial pressure were constantly monitored and recorded every five minutes. The surgical procedure consisted of gas decompression, repositioning, small intestine massage, enterotomy and/or enterectomy. After the recovery of anaesthesia, the horses were kept in the intensive care unit and treated with infusion of Lactated Ringer's solution complemented in ions and/or glucose if needed and removal of the reflux until the intestinal motility resumed. The postoperative medication included enoxaparine (160 mg/horse subcutaneously once a day) and flunixin meglumine (0.25 mg/kg intravenously thrice a day) during at least the first three days, phenylbutazone (2 mg/kg intravenously twice a day) during the first five days and sodium penicillin (20 000 UI/kg intravenously four times a day) and gentamicin (6.6 mg/kg intravenously once a day) during 8–10 days. Additional analgesia was provided as necessary. Gastrointestinal prokinetics were used in cases of postoperative ileus. Antibiotic therapy and other treatments were adapted according to the patient's response to treatment.

The affected portion of the gastrointestinal tract (small or large intestine), the disease process, and the postoperative complications were identified and recorded. The outcome of hospitalisation was categorised as survival at discharge or death.

Blood sampling timing

Venous blood was collected into tubes containing EDTA before surgery, during surgery after correction of the intestinal lesion and during the recovery of anaesthesia. During the postoperative period, samples were taken every four hours during the first four days (from day 0 until day 4) and then every 12 hours until day 6 (144 hour after the surgery) or until euthanasia.

Leukocytes counts

Haematologic analyses were performed at the time of sampling by use of the Medonic CA 530 (Menarini, Zaventem, Belgium). The technique used by this automated impedance cell haematology analyser was validated for the use in horses (Roleff and others 2007).

Statistical analyses

Population 1

Means and CIs of the leukocyte counts were computed on the total of 53 horses per survival and time to obtain the observed blood leukocytes kinetics.

Population 1b

Population 1b consisted of 45 horses from population 1 on which the complete information was available (per protocol analysis) and that survived at least 68 hours postoperatively. A receiver operating characteristic (ROC) analysis (Metz 1978, 2006, Greiner and others 2000, Fawcett 2006, Linden 2006) was performed on the 20 first leukocyte counts (ie, up to 68 hours postoperatively) on these 45 horses.

Under the Bayesian framework, we assumed repeated measures of blood leukocytes are distributed normally as $y_i^t \sim N(\mu_i^t, (\sigma_i^t)^2)$ where y_i^t is the log-transformed leukocytes, the index i is for the horse (i=1, 2, … 45), and the index t is for the observation within horse (t=1, 2, … 20). We verified the normality assumption via the shape of the histogram of the test values, Q-Q plots and numerical methods of normality test, all provided by SAS procedure Univariate (SAS, V.9.1.3, 2003 SAS Institute, Carry, North Carolina, USA). When t=1, $\mu_i^t=\lambda_d^t$ and $\sigma^{2}{}_i^t=(1/\gamma_d^t)$ where d is for the outcome: d=1 if the animal survives until discharged and d=2 if animal dies during hospitalisation. When t > 1, the conditional means and variances are:

$$\mu_i^t = \lambda_d^t + \rho_d^i(\gamma_d^{t-1}/\gamma_d^t)^{0.5}(y_i^{t-1} - \lambda_d^{t-1})$$
$$(\sigma_i^t)^2 = (1/\gamma_d^t)[(1-(\rho_d^i)^2]$$

where ρ_d^i is a correlation parameter between successive measures within each horse (adapted from Muller and others 2009). We chose inverse-gamma priors for the hyper-parameters $\gamma_d^t \sim IG(0.01, 0.01)$, normal priors for $\lambda_d^t \sim N(0.01, 10^{-6})$ and uniform priors for $\rho_d^i \sim U(-1, 1)$. We used estimates of λ_d^t and $\sigma^{2}{}_i^t$ to construct the 20 ROC curves, one for each observation within horse (t). The ROC curves represented the trade-off between the false negative (1–specificity) and false positive (sensitivity) rates for every possible values of log-transformed leukocyte. We computed the sensitivity (at t) as $[\Phi(y_i^t-\lambda_1^t)/\sqrt{(1/\gamma_1^t)}]$, the corresponding specificity as $[\Phi(y_i^t-\lambda_2^t)/\sqrt{(1/\gamma_2^t)}]$, and the area under each ROC curve (Detilleux and others 1999) as $AUC_t=[\Phi(\lambda_1^t-\lambda_2^t)/\sqrt{(1/\gamma_1^t+1/\gamma_2^t)}]$ where Φ is the cumulative distribution of a standard normal variable. Finally, we considered the point of the ROC plot with the largest Youden Index (= sensitivity+specificity–1) as the best cut-off for the period considered. We implemented the model in Openbugs (Lunn and others 2009) and ran three chains of 100,000 rounds with 5000 burn-in rounds with different starting values for γ_d^t, λ_d^t and ρ_d^i. We checked convergence through the Gelman and Rubin (1992) statistics, and visually inspected the trace plots.

Population 2:

The results obtained from population 1b were tested on a second set of 50 horses (named population 2) that underwent colic surgery in similar conditions within a period of 12 months after the end of data acquisition of population 1(b). Horses of population 2 were treated similarly as population 1b, except for sampling intervals which were every eight hours. We used thresholds identified in population 1b to classify horses as 'positive' or 'negative', and computed the observed (or crude) sensitivity and specificity as the observed true positive rate and true negative rate, respectively.

Results

Population 1

This population of 53 horses consisted of 45 Warmbloods, 2 ponies, 2 Arabians, 1 crossbred Arabian, 1 Thoroughbred, 1 Spanish bred and 1 Draft horse. There were 32 mares, 16 geldings and 5 stallions. Their ages ranged from 0.5 years to 20 years, with a median of 8.0 years. Their weight varied from 203 kg to 840 kg (median: 560 kg). Box 1 shows the predominant lesion diagnosed during surgery for each horse of population 1.

Of 53 horses, 12 were non-survivors (euthanased due to severe shock or recurrent/uncontrollable pain). For the horses euthanased

BOX 1: Predominant lesion of each horse of population 1 diagnosed at the time of surgery

- Small intestine (n=22)
  - Volvulus (n=11)
  - Incarceration in the epiploic foramen (n=4)
  - Enteritis (n=2)
  - Strangulation by a lipoma (n=2)
  - Ileocecal intussusception (n=1)
  - Incarceration in a mesenteric defect (n=1)
  - Ileal impaction (n=1)
- Large intestine (n=31)
  - Large colon displacement (n=14, of which 7 nephrosplenic entrapments)
  - Large colon torsion (n=8)
  - Typhlitis/colitis (n=3)
  - Cecocolic intussusception (n=2)
  - Cecal torsion (n=2)
  - Colitis with thromboembolism of the pelvic flexure (n=1)
  - Abdominal wall perforation with peritonitis and typhlitis (n=1)

during hospitalisation, the survival time varied from 0.5 day to 20 days with a median time of 8.0 days.

The kinetics of leukocyte counts according to the outcome are shown in Fig 1. They were significantly different between survivors and non-survivors at 28, 36, 48 and 60 hours postoperatively as shown by the non-overlapping of the CIs. The kinetics of blood leukocytes in survivors is higher than in non-survivors from admission until 80 hours postoperatively.

Population 1b

Bayesian posterior point estimates for the AUC are shown in Fig 2 along with their 95 per cent credibility intervals. All were statistically higher than 50 per cent suggesting log-transformed blood leukocyte count has the ability to correctly classify horses that will survive or not after surgery (better than by chance alone).

The AUCs were not statistically different from each other (credibility intervals overlapped) but the highest AUC was observed at 36 hours postoperatively. The ROC curve with its credibility interval for this time point is shown in Fig 3. The point on this ROC curve at which Youden index was the highest was for a blood leukocyte count=3.9×10^3/mm^3 with a sensitivity=69.97 per cent and a specificity=85.47 per cent (the test was considered positive when a blood leukocyte count ≤3.9×10^3 leukocyte/mm^3 was observed). At 36 hours postoperatively, non-survivor horses were more likely to have a blood leukocyte count ≤3.9×10^3/mm^3 than survivor horses.

In Fig 2, we defined a region for which AUC posterior median was higher than 80 per cent (from 28 to 60 hours postoperatively) as the most interesting region to obtain blood leukocyte thresholds with the best prognostic accuracy. These leukocyte thresholds vary from 3.8 up to 4.9×10^3/mm^3 blood (Table 1). At each hour postoperation, these cut-off values have the best prognostic accuracy (best Youden index) as compared with other blood leukocyte values observed at

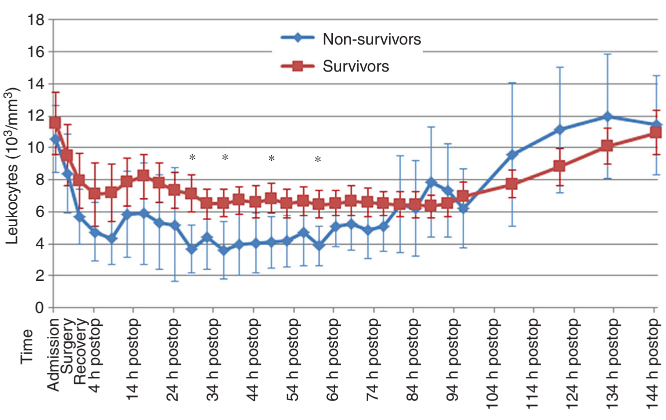

FIG 1: Kinetics of blood leukocyte counts (means in 10^3/mm^3±CIs) according to the outcome, from admission in clinic until 144 hours postoperatively (postop) in population 1. *: significant differences of mean blood leukocyte counts between survivors and non-survivors (as shown by the non-overlapping of the CIs)

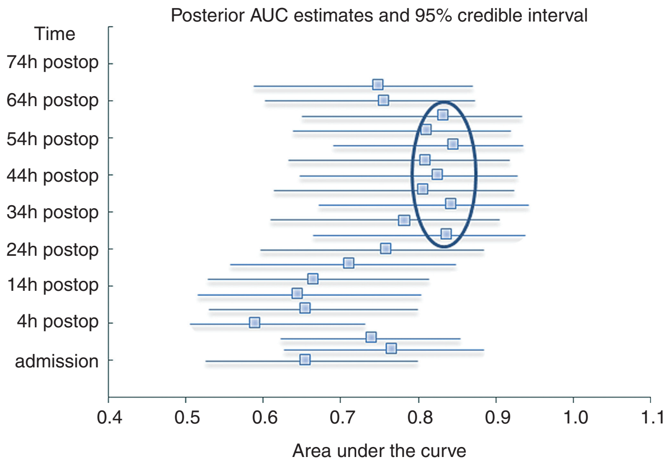

FIG 2: Posterior Bayesian (and 95 per cent credibility interval) estimates of the area under the receiver operating characteristic curves for time from admission to 68 hours postoperatively (postop) in population 1b. The blue lines correspond to the credibility intervals around the AUC posterior medians (squares)

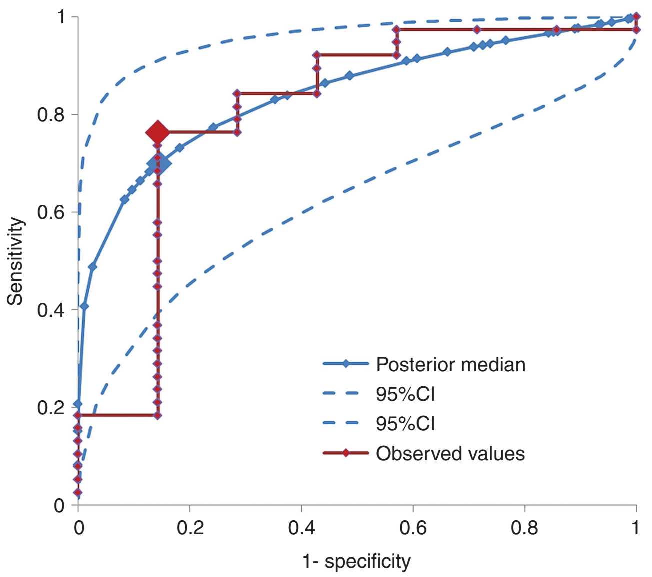

FIG 3: Observed (plain red line) and posterior Bayesian (plain blue line) with its 95 per cent credibility interval (CI-hatched blue line) estimates of the receiver operating characteristic curve at 36 hours postoperatively. The enlarged points are the points with the highest Youden indices

TABLE 1: Sensitivity (per cent), specificity (per cent) and Youden index (per cent) for blood leukocyte counts with the best Youden index from 28 to 60 hours postoperation (population 1b)

Hours post-operatively	Leukocytes (×10^3/mm^3)	Sensitivity	Specificity	Youden index
28	4.1	71.90	80.80	52.70
32	3.8	62.55	81.19	43.74
36	3.9	69.97	85.47	55.44
40	4.1	65.44	84.34	49.78
44	4.5	75.63	73.69	49.32
48	4.4	66.15	81.26	47.41
52	4.9	80.71	71.27	51.98
56	4.2	65.66	82.46	48.12
60	4.5	75.01	75.82	50.83

BOX 2: Predominant lesion of each horse of population 2 diagnosed at the time of surgery

- Small intestine (n=20)
  - Volvulus (n=6)
  - Incarceration in the epiploic foramen (n=1)
  - Strangulation by a lipoma (n=4)
  - Umbilical hernia (Richter) (n=2)
  - Inguinal hernia (n=4)
  - Ileal impaction (n=3)
- Large intestine (n=30)
  - Large colon displacement (n=16, of which 1 nephrosplenic entrapments, and 3 sand impactions)
  - Large colon torsion (n=7)
  - Typhlitis/colitis (n=3)
  - Large colon impaction (n=2)
  - Cecal torsion (n=1)
  - Volvulus of the small colon with foreign body (n=1)

that hour postoperation. For this entire period (28–60 hours postoperatively), we have chosen 3.9×10^3 leukocytes/mm^3 blood as the best cut-off because the AUCs at each time point for the period were not significantly different from each other (Fig 2), because it has the highest specificity and the highest Youden index, and because it is almost the lowest value.

Population 2

This population (50 horses) consisted of 30 Warmbloods, 5 ponies, 2 Trotters, 2 Appaloosas, 2 Frisians, 2 Fjords, 1 Arabian, 1 crossbred Arabian, 1 Quarter horse, 1 Paint horse, 1 Spanish bred, 1 Haflinger and 1 Irish Cob. There were 24 mares, 21 geldings and 5 stallions. Their ages ranged from 0.5 year to 22 years, with a median of 7.5 years. Their weights varied from 145 kg to 728 kg (median: 505 kg). ⚐Box 2 shows the predominant lesion diagnosed during surgery for each horse of population 2.

In this population, 13 out of the 50 horses died or were euthanased during hospitalisation. Using the range of thresholds obtained during the period of 28–60 hours postoperation in population 1b (see Table 1), we computed the sensitivity and specificity for the period of interest in the population 2 (Table 2). The best cut-off was again at blood leukocyte count of 3.9×10^3/mm^3 with a specificity of 75.82 per cent and a sensitivity of 44.12 per cent.

Populations 1b and 2

Therefore, as it was confirmed in the population 2, the value of 3.9×10^3 leukocytes/mm^3 appears to be the best cut-off value during the period of interest (28–60 hours postoperatively) (see Tables 1 and 2). A leukocyte count $\leq3.9\times10^3$/mm^3 during this period can often be related to an unfavourable outcome.

Discussion

The biphasic blood leukocytes kinetics observed in the present study (a decrease followed by an increased leukocytes count) is similar – even if prolonged in time – to the kinetics of human blood leukocytes observed after experimental endotoxaemia in a model of systemic inflammatory response syndrome termed as SIRS (Calvano and Coyle 2012). In our non-survivor horses, this biphasic response was even more accentuated.

While the pathophysiology of SIRS and the events leading to multiple organ dysfunction syndrome and death are not completely understood (Bosmann and Ward 2013), it seems that leukocytes and especially neutrophils could play a crucial role (Weiss and others 2009). At sites of infection or tissue injury, neutrophil lifespan is prolonged for effective host defence (Milot and Filep 2011). Neutrophil apoptosis was found to be inversely proportional to the severity of sepsis and was therefore proposed as a marker of the sepsis severity (Fialkow and others 2006). The kinetic of blood leukocytes according to the outcome described in the present study is thus a further step to improve our knowledge of inflammation related to surgical colic in horses. The leukocytes kinetics may be used to study different treatment protocols aiming to modulate the inflammatory response. Further, with the help of leukocyte kinetics, we can explain the possible deleterious effects of neutrophils by their excessive activation and release of large amounts of inflammatory mediators (ie, cytokines), proteolytic enzymes (ie, elastase, cathepsin, matrix metalloproteinases) and oxidant enzymes (ie, NADPH-oxydases, NO-synthase, myeloperoxidase (MPO)). A significant increase of plasma MPO concentration was yet observed in horses suffering from colic or laminitis (Deby-Dupont and others 1998, Riggs and others 2007, Grulke and others 2008, de la Rebiere de Pouyade and others 2010). Recently, the MPO index was investigated as a diagnostic indicator of systemic inflammation in horses (Schwarz and others 2012).

We found that in our hospital, the horses that did not survive were more likely to present at least one blood leukocyte count $\leq3.9\times10^3$/mm^3 between 28 hours and 60 hours after colic surgery. Even if the sensitivity of the test is lower than 50 per cent in population 2, the specificity equal to, or higher than, 70 per cent in both populations indicates that few errors will be made among survivor horses. Although this cut-off value was computed during the postoperative period, and thus when the surgical expenses have already been invested, it occurred quite early during the postoperative period. It can be used as an additional tool when the final outcome remains unclear in a horse developing severe postoperative complications and helps to avoid prolonging life and hopeless suffering of such horses. Further, this test has economic advantages, as it is inexpensive and it can be useful in avoiding the expensive costs of intensive care in desperate cases.

In the current study, we reported estimates of sensitivity and specificity of blood leukocyte counts. These values are not equivalent to probabilities of mortality/survival, which are positive and negative predictive values. As predictive values depend on the prevalence of the condition, it will be wrong for clinicians to directly apply published predictive values of a study to their own populations, especially when the prevalence of the condition (in our case mortality after colic surgery) in their population differs from the prevalence of conditions in the population to which it is compared (Akobeng 2007). Therefore, clinicians should use the sensitivity and specificity published here in relationship with the prevalence of mortality after colic surgery in their own hospital population. Like this, they will be able to determine positive and predictive values of the test for their own hospital population.

However, a major limitation of the present study is that blood leukocyte count was the only parameter used to evaluate the outcome after surgery, and that clinical and clinicopathological data were not included in our statistical model. Therefore, the blood leukocyte count should be associated with predictors of mortality/survival after colic surgery determined by other studies.

Morton and Blikslager (2002) and Driscoll and others (2008) found that an increased heart rate 24 hours after small or large intestinal resection and anastomosis was significantly associated with non-survival. Postoperative pain, ileus, endotoxemia, septic peritonitis, repeat celiotomy were also associated with postoperative mortality (Morton and Blikslager 2002, Stephen and others 2004, Mair and Smith 2005b, Driscoll and others 2008). Survival was found to be decreased in horses that had suffered from an incarceration in the epiploic foramen (Morton and Blikslager 2002, Proudman and others 2002). Various other parameters have been evaluated for prognosis after colic surgery, with conflicting results for some of them, depending on the study (Mair and others 2007).

Our results do not suggest that blood leukocyte must be counted every four or eight hours postoperatively in every horse after colic surgery, but they can help interpreting the postoperative leukopenia and give a way to take advantage from the blood leukocyte count

TABLE 2: Sensitivity (per cent), specificity (per cent) and Youden index (per cent) for different blood leukocyte counts in the population 2 during the period 28–60 hours postoperation

Leukocytes ($\times10^3$/mm^3)	Sensitivity	Specificity	Youden index
3.9	44.12	75.82	19.94
4.0	44.12	73.63	17.75
4.2	47.06	71.43	18.49
4.4	50.00	64.84	14.84
4.5	50.00	63.74	13.74
4.7	58.82	60.44	19.26
4.9	61.76	56.04	17.80

obtained from the routine haematological profiles after colic surgery. Moreover, they provide evidence for further research to test the value of blood leukocyte counts alongside other parameters in a wider population of horses.

In conclusion, routine values of blood leukocyte counts can be used as an additional prognostic indicator after colic surgery alongside other predictors previously associated with the outcome.

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