Acute phase proteins as diagnostic markers in horses with colic

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Acute phase proteins as diagnostic markers in horses with colic

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1 **Abstract**

2 **Objective** - To investigate the diagnostic potential of acute phase proteins (serum amyloid A
3 (SAA), haptoglobin and fibrinogen) measured in blood and peritoneal fluid for differentiating horses
4 with inflammatory colic (entero-colitis and peritonitis) from those with surgical colic.

5 **Design** - Prospective observational multicenter study.

6 **Setting** - Two university referral hospitals.

7 **Animals** - Horses referred for severe acute abdominal pain to hospital 1 (n=148) or hospital 2
8 (n=78).

9 **Intervention** - Blood and peritoneal fluid samples collected at admission were used for acute
10 phase protein measurement.

11 **Measurements and Main Results** - A multivariable logistic model including clinical parameters
12 (lethargy, rectal temperature > 38°C, normal rectal findings and gastric reflux of 5-10 L) recorded at
13 admission was constructed from hospital 1 data. The ability of the model to correctly differentiate
14 inflammatory from surgical colic was 86% determined as area under the receiver operating
15 characteristic curve (AUC).

16 Adding blood parameters (white blood cell count (WBC), packed cell volume (PCV), total plasma
17 protein, lactate, SAA, haptoglobin and fibrinogen) to the logistic model based on clinical parameters
18 revealed that only SAA, WBC and fibrinogen improved the model. With SAA included in the model no
19 additional blood parameters improved the model, and the final model had an AUC of 90%. Addition of
20 peritoneal fluid parameters (hemolysis, total protein, WBC, SAA, haptoglobin) did not improve the
21 model significantly. When validated in hospital 2 data, the models had a satisfying integrity and
22 diagnostic performance.

23 **Conclusions** – Evaluation of SAA in serum improved the ability to differentiate horses with acute
24 inflammatory colic requiring medical treatment from horses with colic requiring surgical treatment, as it
25 allowed an additional 4% of horses to be correctly classified into medical and surgical cases. Improved
26 differentiation of these two groups of horses with colic may minimize the risk of unnecessary or
27 delayed surgery.

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29 **Words: 297 max 300**

30

31 **Keywords:**

Acute phase proteins, serum amyloid A, haptoglobin, fibrinogen, peritoneal fluid, lactate, white
blood cell count, colic, horse, diagnostic accuracy, surgery

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34 **Abbreviation list**

35	APP	Acute phase proteins
36	AUC	Area under the curve
37	DPJ	Duodenitis-proximal jejunitis
38	Hp	Haptoglobin
39	PF	Peritoneal fluid
40	ROC	Receiver operating characteristic
41	SAA	Serum Amyloid A

42 **Introduction**

43 Horses with acute abdominal pain (colic) have traditionally been categorized into two major groups
44 based on the required treatment namely horses where surgery is required and horses that can be
45 treated medically¹⁻⁶. This categorization is highly relevant because early surgical intervention
46 optimizes the prognosis of the horse⁷. Horses in the acute stages of some inflammatory abdominal
47 diseases such as duodenitis-proximal jejunitis (DPJ), acute colitis^{8,9} and peritonitis¹⁰, often present
48 with the same severe signs of shock, pain, positive gastric reflux and/or changes in peritoneal fluid
49 (PF) as horses with strangulations, displacements or severe impactions requiring surgical intervention.
50 For horses with inflammatory colic, surgery will be an unnecessary burden and should be avoided¹¹.

51 During inflammation, acute phase proteins (APPs) are released in circulation¹². This is also the
52 case in blood and PF of horses with colic, where higher concentrations of APPs are present in
53 inflammatory diseases compared to simple obstructions or strangulations¹³⁻¹⁶. However, the diagnostic
54 performance of APPs in differentiating inflammatory colic from colic that requires surgery has not been
55 evaluated.

56 The objective of this study was to assess the value of APPs in the decision of medical versus
57 surgical treatment in horses referred with severe acute abdominal pain to an equine hospital. A
58 second objective was to evaluate the diagnostic value of adding PF evaluation to the information
59 obtained from clinical examination and measurements of blood biomarkers. The study involved horses
60 from two hospitals. The statistical models were developed based on data from one hospital and

subsequently validated on data from the other hospital. This validation served to evaluate the robustness of the predictive models and to assess if they could be generalized to encompass hospitals with varying horse populations.

Materials and Methods

Study design and population

The study was a prospective observational multicenter study involving horses admitted with severe acute abdominal pain to XX (Hospital 1) from September 2008 to May 2011 and horses admitted to XX (Hospital 2) from August 2009 to December 2010. Horses were excluded if blood samples were not collected at admission, if the horse was less than 1 year old or pregnant with less than 1 month to term, or if a concurrent inflammatory disease unrelated to the abdomen was present (e.g. respiratory infections, hoof abscesses or wounds). Horses treated medically for simple obstructions, horses without a final diagnosis that responded to medical treatment, and horses with gastrointestinal ruptures were excluded from this study. Horses presenting with diarrhea or peritonitis without abdominal pain were also excluded from the study as the primary objective was to evaluate the diagnostic performance of the APPs in a population of horses that present a diagnostic challenge to the clinician.

The study was approved by the ethical boards of both hospitals. All data and samples were collected with the owner's permission as part of the routine diagnostic workup of the cases.

Variables

All horses underwent a full clinical examination immediately after admission, including rectal examination, nasogastric intubation, abdominocentesis, blood-gas analysis, fecal analysis for presence of sand, parasite eggs and larvae. Abdominal ultrasonography was performed in selected cases only. Clinical variables recorded were heart rate, respiratory rate, pain (none, mild, moderate, severe, lethargy), borborygmia (normal, decreased, ceased, increased), gastric reflux (<5L, 5-10L, >10L), rectal findings (normal, impactions, dilatations, displacements), rectal temperature, capillary refill time (CRT), feces (normal, dry, soft, watery, none) and mucous membranes (pink, pale, congested, cyanotic). Blood was sampled for hematologic and serum biochemistry analyses.

88 Variables measured in blood included serum amyloid A (SAA), haptoglobin (Hp), fibrinogen, iron,
89 lactate, total white blood cell counts (WBC), total plasma protein (TPP) and packed cell volume (PCV).
90 Variables measured in PF included SAA, Hp, total protein (TP), WBC and presence or absence of
91 hemolysis. A final diagnosis was established based on all the information collected (excluding SAA,
92 fibrinogen and Hp) and, when available, surgical and post mortem findings. Demographic data, results
93 of the clinical, hematologic, serum biochemical, PF and fecal analyses, pre-admission duration of colic
94 (0-12h, 13-24h, >24h), required treatment (medical, surgical), and disease process (simple
95 obstruction, strangulating obstruction, inflammatory, rupture, other and unknown) were recorded.
96 Inflammatory diseases were defined as horses with DPJ, acute typhlo-colitis and acute peritonitis.
97 Duodenitis-proximal jejunitis was diagnosed as horses with excessive gastric reflux (>20L) over >24h
98 that responded to medical treatment or where no concurrent mechanical obstruction was identified at
99 surgery or necropsy^{17,18}. Acute typhlo-colitis was diagnosed at necropsy and in horses that had
100 severely compromised peripheral perfusion and developed diarrhea. Peritonitis was diagnosed as the
101 primary disease in horses with PF WBC $>10 \times 10^9$ cells/L that responded to medical treatment, or had
102 no apparent cause identified at surgery or post-mortem examination. Horses were assigned to either
103 the medical or surgical group retrospectively by the principle investigator (THP) based on the final
104 diagnosis.

105 *Collection and management of samples*

106 Blood and PF samples were collected and stored as described by Pihl et al.¹⁶. Briefly, unstabilized
107 and citrate-stabilized blood samples and EDTA-stabilized PF samples were centrifuged at 2000 g for
108 10 minutes and supernatants stored at -80°C until analysis for SAA, Hp and fibrinogen. Samples were
109 shipped from hospital 2 to hospital 1 on dry ice with a professional cold chain operator ensuring
110 constant temperature below -80°C.

111 *Laboratory analyses*

112 Packed cell volume and TPP were assessed by means of a Hawkley's microhaematocrit reader^a
113 and refractometry^b respectively. Blood WBC were performed on two highly correlated automated
114 instruments (ADVIA 120^c in hospital 1 and ADVIA 2120^d in hospital 2)¹⁹. Plasma lactate was assessed

115 within 10 minutes of collection by a spectrophotometric blood-gas analyzer^e in hospital 1 and by a
116 handheld lactate analyzer^f in hospital 2. The handheld lactate analyzer had an acceptable correlation
117 ($r=0.75$) with a spectrophotometrically enzymatic kit at lactate concentrations < 10 mmol/L²⁰. Serum
118 amyloid A and Hp analyses of samples from both hospitals were performed in one batch at the
119 laboratory of hospital 1. Serum amyloid A was measured with the LZ SAA immunoturbidometric
120 assay^g in an ADVIA 1800^h as described by Jacobsen et al. (2006)²¹. Haptoglobin was measured with
121 the Phase Range Haptoglobin assayⁱ in an ADVIA 1800^h as described by Pihl et al. (2013)²².
122 Fibrinogen was measured by the Clauss method in an automated coagulometric analyzer ACL 9000^j
123 as described by Andersen et al. (2012). Serum iron was measured by colorimetric spectrophotometry
124 on an ADVIA 1650^a as described by Andersen et al. (2012)²³.

125 *Statistical analyses*

126 The statistical outcome variable was presence or absence of inflammatory colic. Univariable
127 logistic regression analysis of all registered variables from clinical findings, blood and PF analyses
128 was used to identify variables eligible for inclusion in the multivariable models. For each variable the
129 effect of pre-admission duration of colic was tested as an interaction term in both the univariable and
130 multivariable analyses. Variables with $p < 0.20$ in the univariable analyses were included in the
131 multivariable models²⁴. Manual backward elimination, followed by forward selection was used to
132 construct the multivariable models. The criterion for retaining a variable in the final models was $p <$
133 0.15 ²⁵. Dummy variables for each category were constructed for the variables with multiple levels.
134 Rectal temperature and CRT were changed to dichotomous variables (temperature deviation from
135 38°C and CRT to $\leq 2\text{s}$ or $> 2\text{s}$) before entering the statistical models based on the biological definitions
136 of normal values. A 'clinical model' was constructed by including only clinical variables. Variables
137 measured in blood samples were then added to the clinical model; to construct a 'clinical + blood
138 model'. Finally variables measured in PF were added to construct a 'clinical + blood + PF model'. This
139 statistical approach was chosen to reflect the need of increasingly invasive methods to obtain samples
140 (thus a clinically practical approach rather than a pure mathematical approach which would have
141 identified the best markers overall). Data fit was evaluated by max rescaled R^2 and Hosmer and
142 Lemeshow goodness of fit. The influence of single observations on the models was tested by the

residuals and covariate patterns of the regression diagnostics. The diagnostic performance of the resultant models was assessed by area under the receiver operating characteristics (ROC) curve (AUC) and by sensitivity and specificity at an optimal diagnostic cut-off point selected from the ROC-curve. A high specificity was prioritized in order to ensure that the horses identified as having inflammatory colic truly belonged to this group and did not require surgery, as misclassifying a horse with a surgical condition would potentially have more grave implications than vice versa.

In order to assess the robustness, each model was validated on data from hospital 2 in three steps, as described by Wiinberg et al.²⁶: 1) Variables included in the model constructed on data from hospital 1 were tested for significant contribution to the model when applied to data from hospital 2; 2) Variables excluded in the model were re-entered into the model by forward selection and their potential significant contribution to the model was evaluated; 3) Diagnostic performance of the model was assessed by applying the model and the defined cut-off value to data from hospital 2. Demographic data was compared between the two disease groups overall and within each hospital with student's T-test for continuous variables (age and weight) and Chi-square-test for categorical variables (gender, breed) before logistic regression analyses. Confounding in the models was also tested by adding the demographic variables to the final models. Statistical analyses were done with SAS 9.2^k.

Results

Study population

In total, 226 horses with acute severe colic were included in this study. The distribution of horses with inflammatory colic and surgical colic was similar in the two hospitals ($p=0.3$), even though the distribution of specific diagnoses within each disease group varied (Table 1). There was no significant difference between age and gender in the two disease groups overall or within each hospital (Table 2). Horses with inflammatory colic weighed less than horses with surgical colic in hospital 1 and there was significantly more coldblooded horses in the inflammatory group than in the surgical group in hospital 2 (Table 2).

171 *Clinical model*

172 Clinical variables identified by univariate analyses and included into the 'clinical model' were: pain,
173 gastric reflux, rectal findings, feces, temperature above 38.0 °C, borborygmia and CRT (Table 3). Pre-
174 admission duration of colic did not influence any of the clinical variables evaluated. The final 'Clinical
175 model' for prediction of inflammatory colic based on data from hospital 1 included lethargy,
176 temperature increase from 38 °C, gastric reflux 5-10 L and normal rectal findings (Table 54). All
177 variables were positive predictors of inflammatory colic, except gastric reflux 5-10 L which was a
178 negative predictor. The model was not confounded by demographical data (age, gender or breed) nor
179 significantly influenced by single observations. The AUC was 0.86 (95 % CI: 0.79-0.93) (Figure 1).
180 With a selected diagnostic cut-off at $p=0.576$ the diagnostic specificity was 98 % and the sensitivity
181 was 57 %. This means that 2% of horses requiring surgery were incorrectly classified as inflammatory
182 (the false positive rate), while 43% of horses with inflammatory colic were incorrectly classified as
183 requiring surgery (the false negative rate). Given the prevalence of inflammatory colic of 28% in
184 Hospital 1, the positive predictive value of the 'Clinical model' was 93% and the negative predictive
185 value 83% (Table 65).

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186 *Clinical model validation*

187 When the model developed in hospital 1 was evaluated using the population from hospital 2 all
188 variables except gastric reflux 5-10 L were valid predictors. The model had an AUC of 0.75 (95 % CI:
189 0.61-0.89) and at the selected cut-off at 0.576 the specificity was 96 % and sensitivity 31 % (Table
190 65). Gastric reflux >10 L contributed significantly as a positive predictive variable with OR = 8.0 (95 %
191 CI: 1.5-43.6) in hospital 2. The 'Clinical model' including gastric reflux >10L was therefore chosen as
192 the best clinical model for prediction of inflammatory colic (Table 4).

193 *Effect of adding blood variables to the clinical model*

194 Blood variables added to the clinical model were PCV, SAA, fibrinogen, iron and WBC (Table 3).
195 Serum amyloid A, fibrinogen, and WBC (combined with the duration of colic (D), $WBC \cdot D$) all improved
196 the clinical model with AUCs of the new models of 0.90, 0.87 and 0.86 respectively. However, serum

SAA ~~was associated with the greatest improvement in~~ improved the clinical model ~~most~~ and ~~moreover,~~ with SAA in the model (Figure 2). ~~None of the other~~ measured biomarkers ~~measured in blood~~ significantly improved the model (Figure 2). This model was not confounded by demographical data (age, gender or breed) nor significantly influenced by single observations.

This model had an AUC of 0.90 (95 % CI: 0.84-0.96) (Figure 2). With a selected diagnostic cut-off at $p=0.5076$ the diagnostic specificity of the model was 98 % and the sensitivity was 64 % (Table 65). This means that in comparison to the clinical model the same number of horses (2%) was falsely classified as inflammatory, whereas 35% horses instead of 43% were falsely classified as surgical. The positive predictive value of the 'Clinical+blood model' was 93% and the negative predictive value 87%.

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When the model was validated on data from hospital 2, serum SAA was also found to significantly improve the clinical model in hospital 2, and no further variables had significant influence on predicting the probability of inflammatory colic. This model had an AUC of 0.84 (95 % CI: 0.72-0.95) (Figure 2), a specificity of 85 % and a sensitivity of 63 % (Table 54 and 65) in hospital 2.

Figure 3 shows the actual concentrations of SAA in serum in the horses with inflammatory and surgical colic of both hospitals.

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Effect of further adding peritoneal fluid variables to the model

Serum amyloid A was the only PF variable that was useful in differentiating inflammatory colic from surgical colic (Table 3). The duration of colic did not influence SAA in PF. The model was not improved by including SAA in PF. In hospital 2, similar results were obtained; therefore no model including PF variables was constructed.

Discussion

The overall aim of this study was to improve the differentiation between inflammatory and surgical colic, in order to select the optimal treatment regimen as early as possible. Assessment of serum SAA

222 added diagnostic information, and including this marker in the predictive models allowed 4% more
223 horses to be correctly classified as inflammatory or surgical cases.

224 The approach in this study differed from previous diagnostic studies on prediction of need for
225 surgery in two respects. Firstly, to reflect the importance of implications on possible falsely categorized
226 horses, the model in this study aims at detecting horses NOT requiring surgery, as this would be
227 valuable information to the clinician. When establishing and evaluating new diagnostic tools with high-
228 impact outcome in a critical situation, a series of important biases are to be avoided if the results are
229 to be useful²⁷. Secondly, to avoid spectrum and limited-challenge biases only horses with severe colic,
230 which was not easily assigned to either medical or surgical treatment based on the clinical
231 examination alone, were included. This was done in order to evaluate the possible benefit of serum
232 and PF APPs in the clinical setting, where it will be applied, as the trained clinician will most likely only
233 revert to clinicopathological support in the acute decision-making, when the clinical examination fails
234 to give a clear picture. Including horses where assignment to the groups in question was clear on
235 clinical examination could have introduced increased risk of these biases.

236 Validating a model in another dataset is strongly recommended^{28,29} in order to test the integrity and
237 performance of the model in another setting than the one in which it was constructed. The prevalence
238 of inflammatory colic was similar between the two hospitals (28 % for hospital 1 and 22 % for hospital
239 2). Even though the distribution of the specific diagnoses within the two groups of colic varied between
240 hospitals, the integrity of the models was high, demonstrated by the fact that the same variables had
241 significant diagnostic value in both hospitals. Interestingly, the variable "large volume reflux (> 10 L)"
242 was a better predictor in hospital 2 than low volume (5-10 L) reflux. A possible explanation for this is
243 that hospital 2 had more horses with DPJ, a condition characterized by large volume gastric reflux.

244 The clinical variables included in the model are in agreement with earlier studies on factors
245 predicting the need for surgery^{1,2,11}. However, in contrast to other studies that have shown that
246 assessment of PF variables such as total protein and hemolysis may provide useful diagnostic
247 information^{2,4,30}, the present study did not detect any further advantages of adding PF variables to the
248 clinical and blood variables. The reason for this finding probably relates to our inclusion criteria, where
249 only severe colic cases were included. Previous studies included less severe abdominal disease, such
250 as simple large intestinal obstructions, when comparing medical and surgical abdominal conditions.

Obstructions and other mild medical abdominal disease often cause little changes to PF, and studies with these cases included often revealed great differences in composition of PF between horses with medical and surgical colic. The advantage of the design of the present study was that it took into account the clinical situation where abdominocentesis is reserved for cases where no other decision making tools are available.

One limitation to this study was the lack of PF evaluation in all horses and the lack of WBC counts in all PF samples. In addition, only data collected at admission was evaluated, since only a few horses requiring surgery had a second PF sample collected. Changes in PF lactate have recently been reported to be valuable in identifying horses requiring surgery when serial measures were performed³¹. Future studies should therefore include serial measurements of blood and PF biomarkers in order to improve the diagnostic and prognostic performance of the markers.

Other studies evaluating models predicting the need for surgery have generally overestimated the need for surgery^{1,2,30}. The diseases most often misclassified as surgical are those of inflammatory origin. With an AUC of 0.9 in hospital 1 and 0.84 in hospital 2, the number of horses misclassified with the model that included serum SAA was lower than with previous models^{1,2,30}.

Serum Amyloid A has been measured in several studies investigating various inflammatory diseases in horses¹². In this study, serum SAA was found to have significant diagnostic capacity in differentiating inflammatory from surgical colic. Including assessment of serum SAA allowed an additional 4% of horses to be classified correctly as needing either medical or surgical treatment.

Duration of disease has been suggested to be an important factor when evaluating APPs and lactate concentrations in horses with colic¹⁶. Adding pre-admission duration of colic to the model did not change the diagnostic performance of SAA or the model significantly. This is likely the result of SAA being a fast reacting biomarker that is significantly increased in horses with colic already at durations of 5-12 h¹⁶. Fibrinogen, Hp and WBC increase later and thus have a stronger dependency on duration¹⁶. When evaluated as single variables, fibrinogen and WBC had significant diagnostic capacity, but when added to the clinical model they did not perform as well as SAA, despite taking duration of colic into consideration. This might be because of the large span of the duration intervals used in this study or because of the often inaccurate estimation of colic duration given by the owner.

279 Because of the acute nature of equine colic and the need for rapid decision-making, a biomarker
280 will only be useful as a diagnostic marker if the analysis can be performed on a single sample basis,
281 within a short time and at a reasonable price³². Such point of care tests are currently commercially
282 available³³.

283 In conclusion, adding measurement of serum SAA to the clinical assessment of horses with severe
284 colic improved the identification of horses with acute inflammatory colic that did not require surgery.
285 Including SAA in the evaluation of horses with abdominal disease can potentially minimize the risk of
286 unnecessary or delayed surgery, and a 4% improvement in diagnosis is relevant in a specialized
287 referral hospital. Validation of the model on an entirely different colic population showed that the model
288 was valid in different hospital settings.

289

Footnotes

- ^aHawksley Medical and Laboratory Equipment, Lancing, UK
- ^bAtago Sur-Ne Clinical Refractometer, ATAGO CO., LTD, Tokyo, Japan
- ^cADVIA 120, Bayer A/S ,Lyngby, Denmark
- ^dADVIA 2120, Siemens Health Care Diagnostics Inc. , NY, USA
- ^eRadiometer ABL725, Radiometer Medical ApS, Brønshøj, Denmark
- ^fAccusport Blood Lactate Analyzer, Roche Diagnostics, Basel, Switzerland
- ^gEIKEN Chemical Co. Ltd., Tokyo, Japan
- ^hADVIA 1800 Chemistry System, Siemens Health Care Diagnostics Inc., IL, USA
- ⁱTridelta Development Ltd., Ireland
- ^jACL 9000, Instrumentation Laboratory
- ^kSAS Institute SAS Inc., NC, USA

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384 **Figure legends**

385

386 Figure 1.

387 A) The best model with clinical parameters predicting the probability of inflammatory colic in horses
388 referred to a referral clinic (hospital 1) with acute, severe abdominal disease. The model included the
389 clinical variables lethargy, rectal temperature above 38.0 °C, gastric reflux 5-10L, and normal rectal
390 findings. With an area under the receiver operating characteristic (ROC) curve (AUC) of 0.86 the
391 model correctly diagnosed inflammatory colic and surgical colic in 86 % of the horses evaluated. B)
392 Validation of the Clinical model in another referral clinic (hospital 2) identified the same variables as
393 significant predictors (except gastric reflux, where large volume reflux (> 10 L) was superior to low
394 volume reflux (5-10 L)). The AUC is 0.82 in hospital 2.

395

396 Figure 2.

397 A) The best model with clinical and blood parameters constructed in hospital 1 included only SAA in
398 serum in addition to the clinical variables. SAA in serum improved the AUC to 0.90 from 0.86. B) SAA
399 in serum likewise improved the clinical model in hospital 2 increasing the AUC to 0.84 from 0.82. With
400 SAA in serum in the model no further blood or PF variables significantly improved the model.

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402 Figure 3.

403 - Serum amyloid A in serum from 226 horses with inflammatory and surgical colic. The
404 horizontal line depicts the median.

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407 **Tables**

408 **Table 1. Diagnoses of horses included in the study from hospital 1 and 2.**

	Hospital 1	Hospital 2
Inflammatory (%)	42 (28%)	17 (22%)
Duodenitis-proximal jejunitis	8 (19%)	6 (35.3%)
Enterocolitis	18 (43%)	6 (35.3%)
Peritonitis	16 (38%)	5 (29.4%)
Surgical (%)	106 (72%)	61 (78%)
Strangulating obstructions	64 (59%)	25 (41%)
Small intestinal strangulations	32 (50%)	15 (60%)
Verminous thromboembolic infarct	12 (19%)	0 (0.0%)
Large colon torsions or strangulations	20 (31%)	10 (40.0%)
Simple obstructions	40 (38%)	35 (57%)
Ascending colon impactions	6 (15%)	5 (14%)
Descending colon impactions	3 (7.5%)	2 (6%)
Caecum impactions	2 (5%)	1 (3%)
Caecum tympani	0	1 (3%)
Colon displacements without strangulation	22 (55%)	18 (51%)
Small intestinal impactions	7 (17.5%)	8 (23%)
Extra-enteral (testicular torsion)	0	1 (2%)
Miscellaneous	2 (2%)	1 (2%)

410 **Table 2. Demographic data of horses included in the study from hospital 1 and 2.**

	Inflammatory colic	Surgical colic	p-value
Horses (n)	59	167	
Hospital 1	42	106	
Hospital 2	17	61	
Age (years)	10.3 (8.7-11.8)	9.4 (8.6-10.2)	0.3
Hospital 1	10.7 (8.8-12.5)	9.9 (8.9-11.0)	0.5
Hospital 2	9.3 (6.4-12.2)	8.4 (7.4-9.5)	0.6
Weight (kg)	449 (417-481)	495 (478-512)	0.015
Hospital 1	445 (406-485)	502 (477-528)	0.02
Hospital 2	459 (397-521)	482 (465-498)	0.5
Gender (%)			0.8
Hospital 1			0.73
Mares	20 (48%)	45 (42%)	
Stallions	3 (7%)	6 (6%)	
Geldings	19 (45%)	55 (52%)	
Hospital 2			0.65
Mares	6 (35%)	28 (46%)	
Stallions	3 (18%)	7 (11%)	
Geldings	8 (47%)	26 (43%)	
Breeds (%)			0.006
Hospital 1			0.35
"Warm blooded" *	22 (52%)	66 (62%)	
"Cold blooded" §	20 (48%)	40 (38%)	
Hospital 2			0.0014
"Warm blooded" *	10 (59%)	57 (93%)	
"Cold blooded" §	7 (41%)	4 (7%)	

411 **Explanatory note:** Age and weight are given as mean and 95% confidence intervals, all other
 412 variables as numbers of horses and percentages.

413 *: “Warm blooded” includes Warmbloods, Standardbreds, Thoroughbreds, Arabians and Western
414 breeds.
415 §: “Cold blooded” includes Icelandic horses, ponies and draught horses.

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418 **Table 3. The predictive value of clinical variables for inflammatory colic**

	n	AUC	Crude OR	95% CI OR	p-value
infectioninflammation					
Clinical categorical variables					
Duration	135	0.57			0.36
>24	43		1.86	0.80-4.34	
13-24	30		1.34	0.51-3.55	
0-12h	62		Reference	-	
Pain	143	0.71			0.15
Lethargy	25		2.4	0.8-7.0	0.9
Severe	23		<0.001	<0.001->999	0.9
Moderate	17		0.3	0.06-1.5	1.0
Mild	46		1.1	0.4-2.8	0.9
No	32		Reference	-	
Borborygmia	145	0.58			0.18
Increased	6		3.0	0.26-35.3	0.1
Ceased	52		0.61	0.92-4.01	0.3
Decreased	82		0.45	0.07-2.91	0.05
Normal	5		Reference	-	
Gastric reflux	141	0.59			0.093
>10 L	17		0.82	0.27-2.52	0.3
5-10 L	23		0.19	0.04-0.85	0.05
<5 L	101		Reference	-	
Rectal findings	139	0.78			<0.0001
Displacements	37		0.03	0.007-0.12	0.0027
Dilated intestines	51		0.05	0.015-0.17	0.03
Obstipation	25		0.09	0.025-0.34	0.7

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Normal	26		1	-	
Feces	135	0.58			0.13
Soft or watery	16		2.25	0.62-8.14	0.08
Dry or no	93		0.74	0.28-1.93	0.08
Normal	26		Reference	-	
Mucous membranes	140	0.58			0.43
Cyanotic	3		0.79	0.15-4.18	0.6
Red	41		1.78	0.78-4.07	0.1
Pale	22		0.82	0.26-2.54	0.6
Pink	68		Reference	-	
Capillary Refill Time (CRT)	128	0.57			0.16
Prolonged (>2s.)	68		1.75	0.81-3.79	
Normal (≤2s.)	60		Reference		
Clinical continuous variables					
Heart rate (beats/min)	147	0.53	1.004	0.98-1.03	0.71
Temperature -38 (°C)	141	0.64	1.92	1.16-3.16	0.01

419 Explanatory note: An odds ratio (OR) = 1 represents no association, OR >1 represents a predisposing
420 association where as an OR < 1 represents a protective association. The OR describes the increased
421 risk for an increase in one unit of the investigated variable. Area under the curve (AUC) describes the
422 probability of correctly classifying an inflammatory case as such (1.00= 100%, 0.5= no better than
423 chance).

424 **Table 4. The predictive value of blood and peritoneal fluid variables for inflammatory colic**

	n	AUC	Crude OR	95% CI OR	p-value
<u>infectioninflammation</u>					
Blood variables					
Packed cell volume (%)	147	0.61	1.04	1.00-1.08	0.033
Plasma protein (g/L)	146	0.53	0.99	0.96-1.02	0.47
WBC (10^9 cells/L)	128	0.58	0.93	0.83-1.04	0.18
Lactate (mmol/L)	146	0.50	1.04	0.96-1.13	0.33
Serum amyloid A (mg/L)	148	0.69	1.001	1.00-1.001	0.0005
Haptoglobin (mg/L)	147	0.53	1.000	1.00-1.00	0.36
Fibrinogen (g/L)	124	0.61	1.30	1.04-1.64	0.023
Iron (μ mol/L)	125	0.62	0.97	0.93-1.01	0.09
Peritoneal fluid variables					
Hemolysis	135	0.53			0.47
Hemolysis	39		0.73	0.31-1.73	
No hemolysis	96		Reference	-	
WBC (10^9 cells/L)	58	0.68	1.002	0.99-1.01	0.60
Total protein (g/L)	140	0.52	1.002	0.98-1.026	0.86
Serum amyloid A (mg/L)	134	0.62	1.001	1.00-1.003	0.0087
Haptoglobin (mg/L)	135	0.52	1.000	1.00-1.001	0.51

425 Explanatory note: An odds ratio (OR) = 1 represents no association, OR >1 represents a predisposing
 426 association where as an OR < 1 represents a protective association. The OR describes the increased
 427 risk for an increase in one unit of the investigated variable. Area under the curve (AUC) describes the
 428 probability of correctly classifying an inflammatory case as such (1.00= 100%, 0.5= no better than
 429 chance).

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430 | Table 54. Results of multivariable logistic regression analysis showing the association
431 | between inflammatory colic and variables included in the ‘clinical model’ and ‘clinical+blood
432 | model’ based on data from hospital 1 and validated in hospital 2.

	n	AUC	Adjusted OR	95% CI Adj. OR	p-value
Clinical model	141	0.86			
Normal rectal findings			38.5	7.4-199	
Lethargy			5.6	1.4-22.7	0.02
Temperature >38 (°C)			1.8	1.0-3.5	0.06
Gastric reflux 5-10L			0.06	0.005-0.7	0.03
Gastric reflux >10L			1.5	0.4-5.2	0.6
Clinical + blood model	141	0.90			
Normal rectal findings			37.3	6.9-202	<0.001
Lethargy			6.7	1.6-28.4	0.01
Temperature >38 (°C)			1.7	0.9-3.3	0.08
Gastric reflux 5-10L			0.07	0.06-0.7	0.03
Gastric reflux >10L			1.9	0.5-7.3	0.3
Serum amyloid A in serum (100 mg/L)			1.06	1.01-1.1	0.01

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433 | Explanatory note: Area under the ROC curve (AUC) describes the probability of correctly classifying
434 | an inflammatory case as such (1.00= 100%, 0.5= no better than chance). An odds ratio (OR) =
435 | 1 represents no association, OR >1 represents a predisposing association where as an OR < 1
436 | represents a protective association. For continuous variables the OR describes the increased risk for
437 | an increase in one unit of the investigated variable. For SAA the OR is given per 100mg/L increase.

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440 **Table 65. Diagnostic performance of the 2 models developed in hospital 1 and validated in hospital 2.**

	AUC	95%CI	Se (%)	Sp (%)	FPR (%)	PPV (%)	NPV (%)	LR+
Clinical model*								
Hospital 1	0.86	0.79-0.93	57	98	2	93	85	28.5
Hospital 2	0.82	0.71-0.93	38	90	10	52	84	3.89
Clinical + Blood model[§]								
Hospital 1	0.90	0.84-0.96	64	98	2	93	87	32
Hospital 2	0.84	0.72-0.95	63	85	15	54	89	4.2

441 Explanatory note::

442 *Clinical model' (Selected cut off= 0.5633 for a positive test):

443 $Y = -0.094 + 0.86 \times \text{lethargy} + 0.59 \times (\text{temperature} - 38.0) + (-1.38 \times \text{gastric reflux } 5-10 \text{ L}) + 0.19 \times \text{gastric reflux } >10 \text{ L} + 1.82 \times \text{normal}$
 444 rectal finding.

445 [§]Clinical+ blood model (Selected cut off =0.5076 for a positive test):

446 $Y = -0.23 + 0.95 \times \text{lethargy} + 0.56 \times (\text{temperature} - 38.0) + (-1.34 \times \text{gastric reflux } 5-10 \text{ L}) + 0.33 \times \text{gastric reflux } >10 \text{ L}$
 447 $+ 1.81 \times \text{Normal_rectal} + 0.00061 \times \text{SAAserum.}$

448 AUC = the percentages of horses correctly classified as diseased (inflammatory) or not (surgical) by the test.

449 Se = Sensitivity = Detection rate = the proportion of disease positives (inflammatory) correctly classified by the test

450 Sp = Specificity = the proportion of disease negative (surgical colic) correctly classified by the test.

451 FPR = False positive rate = $(1 - \text{sp})$ = proportion of horses falsely classified as diseased (inflammatory) by the test.

452 PPV = Positive predictive value = the probability that a positive test is reflecting presence of disease (inflammatory).

453 NPV = Negative predictive value = the probability that a negative test is reflecting absence of the disease (surgical).

454 LR+ = Likelihood ratio = the likelihood ratio of a positive test results in an animal with disease (inflammatory)

Figure 1A) The best model with clinical parameters predicting the probability of inflammatory colic in horses referred to a referral clinic (hospital 1) with acute, severe abdominal disease. The model included the clinical variables lethargy, rectal temperature above 38.0 °C, gastric reflux 5-10L, and normal rectal findings. With an area under the receiver operating characteristic (ROC) curve (AUC) of 0.86 the model correctly diagnosed inflammatory colic and surgical colic in 86 % of the horses evaluated.
159x159mm (300 x 300 DPI)

Figure 1B) Validation of the Clinical model in another referral clinic (hospital 2) identified the same variables as significant predictors (except gastric reflux, where large volume reflux (> 10 L) was superior to low volume reflux (5-10 L)). The AUC is 0.82 in hospital 2.
159x159mm (300 x 300 DPI)

Figure 2A) The best model with clinical and blood parameters constructed in hospital 1 included only SAA in serum in addition to the clinical variables. SAA in serum improved the AUC to 0.90 from 0.86.
159x159mm (300 x 300 DPI)

Figure 2B) SAA in serum likewise improved the clinical model in hospital 2 increasing the AUC to 0.84 from 0.82. With SAA in serum in the model no further blood or PF variables significantly improved the model.
159x159mm (300 x 300 DPI)

Serum amyloid A in serum from 226 horses with inflammatory and surgical colic. The horizontal line depicts the median.
85x91mm (600 x 600 DPI)