Acute phase proteins as diagnostic markers in horses with colic

Tina H. Pihl*, DVM, PhD; Elrien Scheepers, BVSc (Hons), MSc (Vet Science); Macarena Sanz, DVM, MS, DACVIM; Amelia Goddard, BVSc (Hons), MMed Vet (KLD); Patrick Page, BVSc (Hons), MMed Vet (Equine Medicine); Nils Toft , Cand Sci, PhD; Mads Kjelgaard-Hansen, DVM, PhD ; Pia H Andersen, DVM, PhD, DVSc and Stine Jacobsen DVM, PhD

From the University of Copenhagen, Faculty of Health and Medical Sciences, Department of Large Animal Sciences, Medicine and Surgery (Pihl, Andersen, Jacobsen) and Central Laboratory (Kjelgaard-Hansen), Copenhagen, Denmark; and the University of Pretoria, Faculty of Veterinary Science, Department of Companion Animal Clinical Studies, Pretoria, South Africa (Scheepers, Sanz, Goddard, Page, Toft)

*Please address correspondence and offprint requests to: Dr. Tina Holberg Pihl, Højbakkegård allé 5, 2630 Tåstrup, Denmark. E-mail: thpi@sund.ku.dk

The study was funded by a Danish Government PhD grant and by the University of Pretoria from the Department of Companion Animal Clinical Studies Research Fund and the Faculty of Veterinary Science Research Fund. Additional funding was provided by the Danish funds; The Danish Horse Levy Fund and The Kustos Foundation of 1881.

The authors declare no conflicts of interest.

Presented in part at the European College of Veterinary Surgeons Annual Scientific Meeting, Rome, July 2013, and at the Association of American Equine Practitioners Annual Convention, Nashville, December 2013.

Running title: Acute phase proteins in horses with colic

Abbreviations

APP acute phase proteins

AUC area under the curve

CI confidence interval

CRT capillary refill time

DPJ duodenitis-proximal jejunitis

Hp haptoglobin

OR odds ratio

PF peritoneal fluid

SAA serum amyloid A

TPP total plasma protein concentration

Abstract

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

Design: Prospective observational multicenter study.

Setting: Two university referral hospitals.

Animals: Horses referred for severe acute abdominal pain to Hospital 1 (n = 148) or Hospital 2 (n = 78).

Intervention: Blood and peritoneal fluid samples collected at admission were used for acute phase protein concentration measurement.

Measurements and Main Results: A multivariable logistic model including clinical parameters (lethargy, rectal temperature > 38°C [100.4°F], normal rectal examination findings, and gastric reflux of 5 – 10 L) recorded at admission was constructed from Hospital 1 data. The ability of the model to correctly differentiate inflammatory from surgical colic was 86% determined as area under the receiver operating characteristic curve.

Adding blood parameters (WBC; PCV; total plasma protein [TPP], lactate, SAA, haptoglobin, and fibrinogen concentrations) to the logistic model based on clinical parameters revealed that only WBC and SAA and fibrinogen concentrations improved the model. With SAA included in the model no additional blood parameters improved the model, and the final model had an area under the curve of 90%. Addition of peritoneal fluid parameters (hemolysis, total protein concentration, WBC, SAA or haptoglobin concentrations) did not improve the model. When validated in Hospital 2 data, the models had good integrity and diagnostic performance.

Conclusions: Evaluation of SAA in serum improved the ability to differentiate horses with acute inflammatory colic requiring medical treatment from horses with colic requiring surgery, as it allowed an additional 4% of horses to be correctly classified into medical and surgical cases. Improved differentiation of these 2 groups of horses with colic may minimize the risk of unnecessary or delayed surgery.

Keywords: diagnostic marker, equine, lactate, peritoneal fluid, serum amyloid A, white blood cell count

Introduction

Colic in horses is traditionally categorized as either surgical or medical in nature.[1-6] This categorization is important because early surgical intervention improves prognosis in horses that require surgery.[7] Horses with some inflammatory abdominal conditions that are generally treated medically, such as duodenitis-proximal jejunitis (DPJ), acute colitis,[8,9] and peritonitis,[10] often present with the same severe signs of shock, pain, positive gastric reflux, or changes in peritoneal fluid (PF) as horses with strangulations, displacements, or severe impactions that require surgical intervention. For horses with inflammatory colic, surgery would be unnecessary and should be avoided.[11]

During inflammation, acute phase proteins (APPs) are released into circulation.[12] Acute phase proteins are present in blood and PF of horses with colic, and higher concentrations of APPs are present in inflammatory diseases than in simple obstructions or strangulations.[13-16] However, the diagnostic performance of blood and PF APP concentrations in differentiating inflammatory from surgical colic has not been evaluated.

The objective of this investigation was to assess the value of blood APP concentrations as markers of medical versus surgical colic in horses referred with severe acute abdominal pain to an equine hospital. A second objective was to evaluate the diagnostic value of adding PF evaluation to the information obtained from clinical examination and measurements of blood biomarkers. The study involved horses from 2 hospitals. The statistical models were developed based on data from 1 hospital and subsequently validated on data from the other hospital. This validation served to evaluate the robustness of the predictive models and to assess whether they could be generalized to hospitals with different horse populations.

Materials and Methods

Study design and population

This prospective, observational, multicenter study included horses admitted with severe acute abdominal pain to The University Hospital for Large Animals in Copenhagen, Denmark(Hospital 1) from September 2008 to May 2011 and horses admitted to The Equine Clinic at the University of Pretoria, South Africa (Hospital 2) from August 2009 to December 2010. Horses were excluded if blood samples were not collected at admission, if the horse was < 1 year old or pregnant with < 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 Evaluated

All horses underwent a full clinical examination immediately after admission, including rectal examination, nasogastric intubation, abdominocentesis, blood gas analysis, and 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), borborygmi (normal, decreased, ceased, increased), gastric reflux (< 5L, 5 – 10L, > 10L), rectal examination findings (normal, impaction, dilatation, displacement), rectal

temperature, capillary refill time (CRT), feces (normal, dry, soft, watery, none) and mucous membrane color (pink, pale, congested, cyanotic). Blood was sampled for hematologic and serum biochemistry analyses. Variables measured in blood included total WBC; PCV and lactate concentration, serum amyloid A (SAA), haptoglobin (Hp) and, iron in serum, and TPP and fibrinogen concentrations in plasma;. Variables measured in PF included SAA, Hp, and total peritoneal fluid protein concentrations; WBC; and presence or absence of hemolysis (orange color after centrifugation). A final diagnosis was established based on all the information collected, including surgical and post mortem findings when available, without considering SAA, fibrinogen, and Hp data. Demographic data; results of the clinical, hematologic, serum biochemical, PF, and fecal analyses; pre-admission duration of colic (0 – 12h, 13 – 24h, > 24h); medical versus surgical treatment requirement; and disease process (simple obstruction, strangulating obstruction, inflammatory, rupture, other, and unknown) were recorded. Inflammatory diseases were defined as horses with DPJ, acute typhlo-colitis, and acute peritonitis. Duodenitis-proximal jejunitis was diagnosed in horses with excessive gastric reflux (> 20L) over > 24h that responded to medical treatment or in which no mechanical obstruction was identified at surgery or necropsy.[17,18] Acute typhlo-colitis was diagnosed at necropsy and in horses that had severely compromised peripheral perfusion that developed diarrhea. Peritonitis was diagnosed as the primary disease in horses with PF WBC > 10 x 10^9 cells/L (> 10 x 10^3 cells/µL) that responded to medical treatment, or had no apparent cause identified at surgery or post-mortem examination. Horses were assigned to either the medical or surgical group retrospectively by the principal investigator (THP) based on the final diagnosis.

Collection and management of samples

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

Laboratory analyses

Packed cell volume and TPP were assessed by means of a Hawkley's microhaematocrit reader[a] and refractometry[b] respectively. Blood WBC counts were performed on 2 highly correlated automated instruments (ADVIA 120[c] at Hospital 1 and ADVIA 2120[d] at Hospital 2).[19] Blood lactate was assessed within 10 minutes of collection by a spectrophotometric blood gas analyzer[e] at Hospital 1 and by a handheld lactate analyzer[f] at Hospital 2. The handheld lactate analyzer had an acceptable correlation (r = 0.75) with a spectrophotometrically enzymatic kit at lactate concentrations < 10 mmol/L.[20] Serum amyloid A and Hp analyses of serum and PF samples from both hospitals were performed in 1 batch at the laboratory of Hospital 1. Serum amyloid A was measured with the LZ SAA immunoturbidometric assay[g] in an ADVIA 1800[h] as previously described.[21] Haptoglobin was measured with the Phase Range Haptoglobin assay[i] in an ADVIA 1800[h] as previously described.[22] Plasma ibrinogen was measured by the Clauss method in an automated coagulometric analyzer ACL 9000[j] as previously described.[23] Serum iron was measured by colorimetric spectrophotometry on an ADVIA 1650[a] as previously described.[23]

Statistical analyses

The statistical outcome variable was presence or absence of inflammatory colic. Univariable logistic regression analysis of all registered variables from clinical findings, blood, and PF analyses was used to identify variables eligible for inclusion in the multivariable models. For each variable the effect of pre-admission duration of colic was tested as an interaction term in both the univariable and multivariable analyses. Variables with $P < 0.20$ in the univariable analyses were included in the multivariable models.[24] Manual backward elimination followed by forward selection was used to construct the multivariable models. The criterion for retaining a variable in the final models was $P < 0.15$.[25] Dummy variables for each category were constructed for the variables with multiple levels. Rectal temperature and CRT were changed to dichotomous variables (temperature deviation from 38°C and CRT to ≤ 2s or > 2s) before entering the statistical models based on the biological definitions of normal values. A clinical model was constructed by including only clinical variables. Variables measured in blood samples were then added to the clinical model to construct a "clinical + blood" model. Finally variables measured in PF were added to construct a "clinical + blood + PF" model. This statistical approach was chosen to reflect the need of increasingly invasive methods to obtain samples (thus a clinically practical approach rather than a pure mathematical approach, which would have identified the best markers overall). Data fit was evaluated by max rescaled R^2 and Hosmer and 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 curve (AUC) and by sensitivity and specificity at an optimal diagnostic cut-off point selected from the receiver operating characteristics 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 robustness, each model was validated using data from Hospital 2 in 3 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 were compared between the 2 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] The cutoff for statistical significance was $P < 0.05$.

Results

Study population

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

Table 1. Diagnoses of horses included in the study from Hospitals 1 and 2.

	Hospital 1 **n, (%)**	**Hospital 2** **n, (%)**
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%)

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

	Inflammatory colic	**Surgical colic**	***P*-value**
Horses (n)	59	167	0.34
Hospital 1	42 (28%)	106 (72%)	
Hospital 2	17 (22%)	61 (78%)	
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	10 (59%)	57 (93%)	

blooded"*		
"Cold blooded"§	7 (41%)	4 (7%)

Age and weight are given as mean and 95% confidence intervals, all other variables as numbers of horses and percentages." * Warmbloods, Standardbreds, Thoroughbreds, Arabians, and Western breeds. § Icelandic horses, ponies, and draught horses.

Clinical model

Clinical variables identified by univariate analysis and included in the clinical model were pain, gastric reflux, rectal examination findings, feces, temperature > 38.0°C, borborygmi, and CRT (Table 3). Pre-admission duration of colic did not influence any of the clinical variables evaluated. The final clinical model for prediction of inflammatory colic based on data from Hospital 1 included lethargy, rectal temperature increase from 38°C, gastric reflux 5 – 10 L, and normal rectal examination findings (Table 4). All variables were positive predictors of inflammatory colic except for gastric reflux of 5 – 10 L, which was a negative predictor. The model was not confounded by demographic data (age, gender, or breed) nor was it significantly influenced by single observations. The AUC was 0.86 (95% confidence interval [CI]: 0.79 – 0.93; Figure 1). With a selected diagnostic cut-off at p = 0.576 the diagnostic specificity was 98% and the sensitivity was 57%. This means that 2% of horses requiring surgery were incorrectly classified as having nonsurgical disease (the false positive rate), while 43% of horses with inflammatory colic were incorrectly classified as requiring surgery (the false negative rate). Given the prevalence of inflammatory colic of 28% in Hospital 1, the positive predictive value of the clinical model was 93% and the negative predictive value 83%.

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

Variable	n	AUC	Crude OR inflammation	95% CI	*P*-value
Categorical clinical 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	–	
Borborygmi	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
Normal	26		1	–	

Feces	135	0.58			0.13
Soft or watery	16		2.25	0.62 – 8.14	0.08
Dry or none	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	–	
CRT	128	0.57			0.16
Prolonged (> 2 s)	68		1.75	0.81 – 3.79	
Normal (≤ 2 s)	60		Reference		
Continuous clinical variables					
Heart rate (beats / minute)	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**

An OR = 1 represents no association, OR > 1 represents a predisposing association, and an OR < 1 represents a protective association. The OR describes the increased risk for an increase in 1 unit of the investigated variable. The AUC describes the probability of correctly classifying an inflammatory case as such (1.00 = 100%, 0.5 = no better than chance). AUC, area under the curve; CI, confidence interval; CRT, capillary refill time; OR, odds ratio; s, seconds.

Table 4. Results of multivariable logistic regression analysis showing the association between inflammatory colic and variables included in the clinical model and "clinical + blood" model based on data from Hospital 1 and validated at Hospital 2.

	n	AUC	Adjusted OR	95% CI Adj. OR	*P*-value
Clinical model	11	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 – 10 L			0.06	0.005 – 0.7	**0.03**
Gastric reflux > 10 L			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 – 10 L			0.07	0.06 – 0.7	**0.03**
Gastric reflux > 10 L			1.9	0.5 – 7.3	0.3
SAA concentration in serum (100 mg/L)			1.06	1.01 – 1.1	**0.01**

The AUC describes the probability of correctly classifying an inflammatory case as such (1.00 = 100%, 0.5 = no better than chance). An OR = 1 represents no association, OR > 1 represents a predisposing association, and an OR < 1

represents a protective association. For continuous variables, the OR describes the increased risk for an increase in 1 unit of the investigated variable. For SAA, the OR is given per 100 mg/L increase. AUC, area under the curve; OR, odds ratio; ROC, receiver operating characteristic curve; SAA, serum amyloid A.

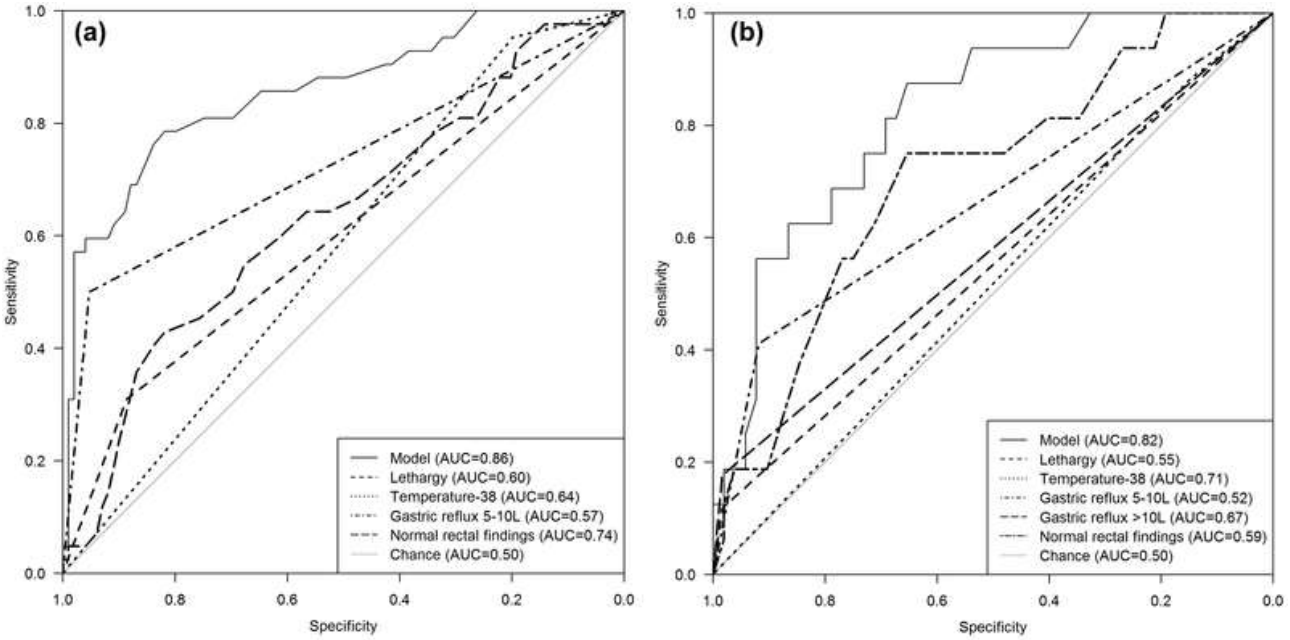


Figure 1. **A)** The best model with clinical parameters predicting the probability of inflammatory colic in horses seen at a referral clinic (Hospital 1) with acute, severe abdominal disease. The model included the clinical variables lethargy, rectal temperature > 38.0°C, gastric reflux 5 – 10 L, and normal rectal examination findings. With an AUC of 0.86, the model correctly diagnosed inflammatory colic and surgical colic in 86% of the horses evaluated. **B)** Validation of the clinical model at a second referral clinic (Hospital 2) identified the same variables as significant predictors with the exception of gastric reflux, in that large volume reflux (> 10 L) was superior to low volume reflux (5 – 10 L) at Hospital 2. The AUC is 0.82 for Hospital 2. AUC, area under the receiver operating characteristic curve.

Clinical model validation

When the model developed at Hospital 1 was evaluated using the population from Hospital 2, all variables except 'low volume gastric reflux (5 – 10 L)' were valid predictors. The model had an AUC of 0.75 (95% CI: 0.61 – 0.89) and at the selected cut-off of 0.576, the specificity was 96% and sensitivity 31% . 'Large volume gastric reflux (> 10 L)' contributed significantly as a positive

predictive variable with odds ratio (OR) of 8.0 (95% CI: 1.5 – 43.6) at Hospital 2. The variable 'Large volume gastric reflux (> 10 L)'was therefore added to the clinical model and a diagnostic cut-off was set at 0.5633 for the final clinical model for prediction of inflammatory colic. Performance of the final validated clinical model is shown in table 5.

Table 5. Diagnostic performance of the 2 models developed at Hospital 1 and validated at 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

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

Y= –0.094 + 0.86*lethargy + 0.59*(temperature – 38.0) + (-1.38*gastric reflux 5 – 10 L) + 0.19*gastric reflux > 10 L + 1.82*normal rectal examination findings.

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

Y= –0.23 + 0.95*lethargy+ 0.56* (temperature – 38.0) + (–1.34*gastric reflux 5 – 10 L) + 0.33*gastric reflux > 10 L +1.81*Normal rectal examination findings + 0.00061*SAA, serum.

AUC, area under the curve, indicates the percentages of horses correctly classified as diseased (inflammatory) or not (surgical) by the test. Se, sensitivity, is the detection rate, which is the proportion of disease positives (inflammatory)

correctly classified by the test. Sp, specificity, which is the proportion of disease negative (surgical colic) correctly classified by the test. FPR, false positive rate, which is calculated as (1 – Sp), and represents the proportion of horses falsely classified as diseased (inflammatory) by the test. PPV, positive predictive value, which is the probability that a positive test is reflecting presence of disease (inflammatory). NPV, negative predictive value, which is the probability that a negative test reflects absence of the disease (surgical). LR+, likelihood ratio, which is the likelihood ratio of a positive test results in an animal with disease (inflammatory).

Effect of adding blood variables to the clinical model

Blood variables added to the clinical model were PCV; SAA, fibrinogen, and iron concentrations; and WBC count (Table 6). Serum amyloid A concentration, fibrinogen concentration, and WBC count (combined with the duration of colic (D), WBC*D) all improved the clinical model with AUCs of the new models of 0.90, 0.87, and 0.86 respectively. However, SAA concentration was associated with the greatest improvement in the clinical model and moreover, with SAA in the model none of the other measured biomarkers significantly improved the model (Figure 2). This model was not confounded by demographic data (age, gender, or breed) nor was it significantly influenced by single observations.

This “clinical + blood” 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 6). In comparison to the clinical model, the same number of horses (2%) were falsely classified as nonsurgical, 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%.

Table 6. The predictive value of blood and peritoneal fluid variables for inflammatory colic

	n	AUC	Crude OR inflammation	95% CI	*P*-value
Blood variables					
PCV (%)	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 (10^9cells/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 (µmol/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 (10^9cells/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

An OR = 1 represents no association, OR > 1 represents a predisposing association, and an OR < 1 represents a protective association. The OR describes the increased risk for an increase in 1 unit of the investigated variable. The AUC describes the probability of correctly classifying an inflammatory case as such (1.00 = 100%, 0.5 = no better than chance). AUC, area under the curve; CI, confidence interval; OR, odds ratio.

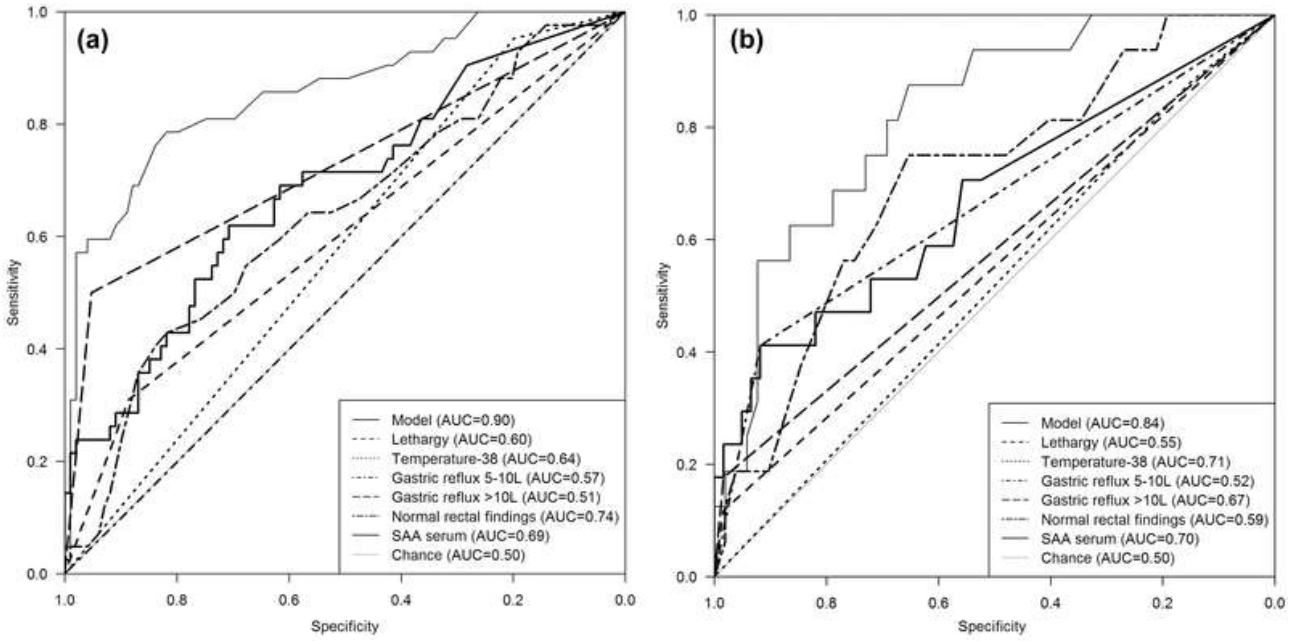
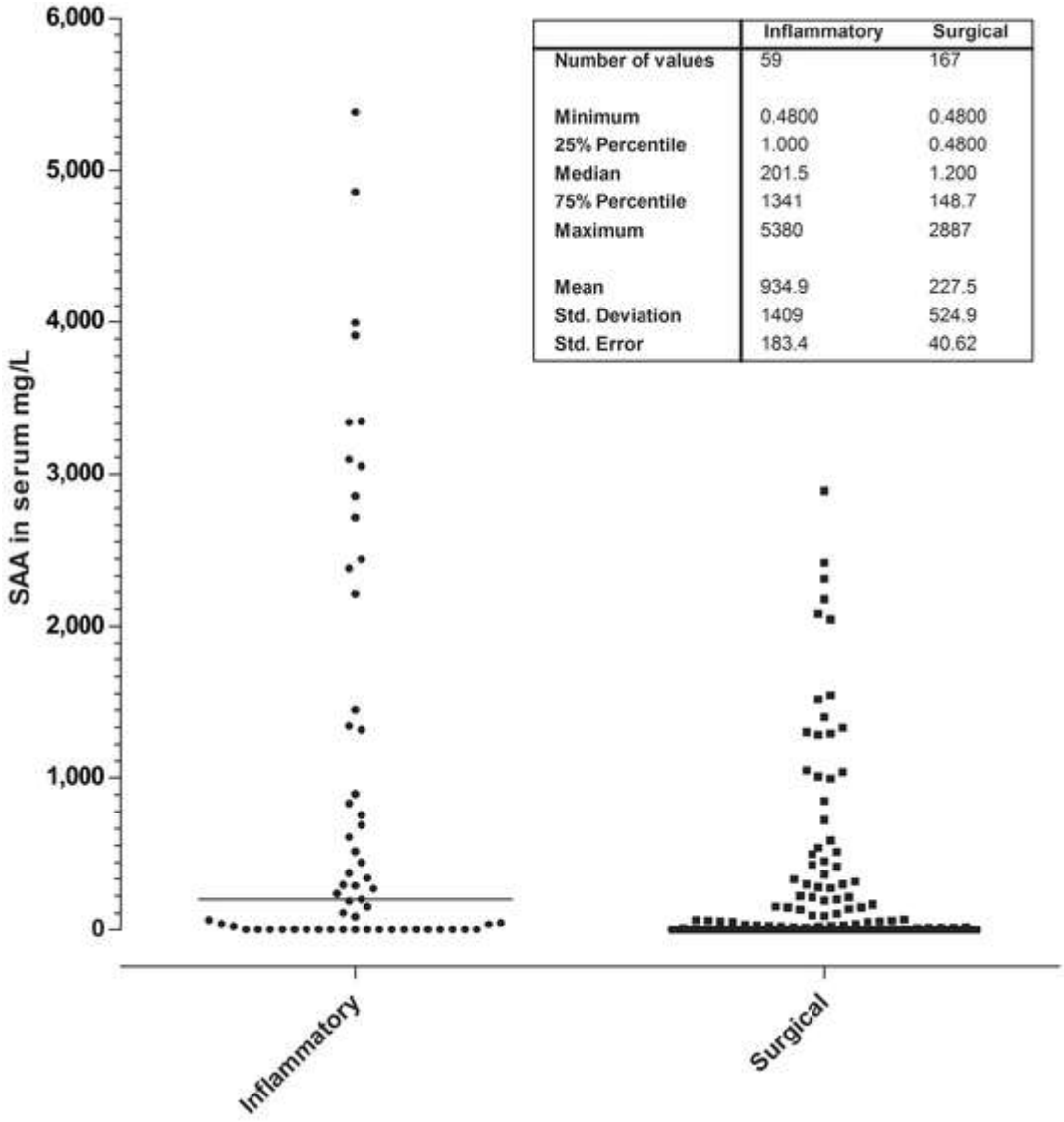


Figure 2. A) The best model with clinical and blood parameters constructed at Hospital 1 included only SAA concentration in serum in addition to the clinical variables. SAA concentration in serum improved the AUC to 0.90 from 0.86. **B)** SAA concentration in serum likewise improved the clinical model at Hospital 2, increasing the AUC to 0.84 from 0.82. With SAA concentration in serum included in the model, no further blood or peritoneal fluid variables significantly improved the model. AUC, area under the receiver operating characteristic curve; SAA, serum amyloid A.

When the model was validated using data from Hospital 2, SAA in serum 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% in Hospital 2 (Table 5). Figure 3 shows the concentrations of SAA in serum in the horses with inflammatory and surgical colic from both hospitals.



	Inflammatory	Surgical
Number of values	59	167
Minimum	0.4800	0.4800
25% Percentile	1.000	0.4800
Median	201.5	1.200
75% Percentile	1341	148.7
Maximum	5380	2887
Mean	934.9	227.5
Std. Deviation	1409	524.9
Std. Error	183.4	40.62

Figure 3. Serum amyloid A concentration in serum from 226 horses with inflammatory and surgical colic. The horizontal line depicts the median. SAA, serum amyloid A; Std., standard.

Effect of adding peritoneal fluid variables to the model

Serum amyloid A concentration was the only PF variable that was useful in differentiating inflammatory colic from surgical colic (Table 6). The duration of colic did not influence SAA concentration in PF. The model was not improved by including SAA concentration in PF. In

Hospital 2, similar results were obtained; therefore, no model including PF variables was developed.

Discussion

The main goal of this investigation was to improve the clinician's ability to differentiate between inflammatory and surgical colic, in order to select an appropriate treatment regimen as early as possible. Assessment of SAA concentration in serum added diagnostic information, and including this marker in the predictive models allowed 4% more horses to be correctly classified as inflammatory or surgical cases.

The approach in this investigation differed from previous diagnostic studies on prediction of need for surgery in 2 respects. First, to reflect the gravity of incorrectly classifying surgical colic cases as nonsurgical, the model in this study aims to detect horses *not* requiring surgery. When establishing and evaluating new diagnostic tools with high-impact outcome in a critical situation, a series of important biases should be avoided if the results are to be useful.[27] Second, to avoid spectrum and limited-challenge biases, only horses with severe colic that was not easily assigned to either medical or surgical treatment based on the clinical examination alone were included. This was done in order to evaluate the possible benefit of serum and PF APP concentrations in the clinical setting, as the trained clinician will most likely revert to clinicopathological support in acute decision-making only when the clinical examination fails to give a clear picture. Including horses where assignment to the groups in question was clear on clinical examination could have increased risk of these biases.

Validating a model in another dataset is strongly recommended[28,29] in order to test the integrity and performance of the model in a different setting than the one in which it was constructed. The

prevalence of inflammatory colic was similar between the 2 hospitals (28% for Hospital 1 and 22% for Hospital 2, p=0.34). Even though the distribution of the specific diagnoses within the 2 groups of colic varied between hospitals, the integrity of the models was high, demonstrated by the fact that the same variables had significant diagnostic value in both settings. Interestingly, the variable "large volume gastric reflux (> 10 L)" was a better predictor in Hospital 2 than low volume (5 – 10 L) gastric reflux. A possible explanation for this discrepancy is that Hospital 2 had more horses with DPJ, a condition characterized by large volume gastric reflux.

The clinical variables included in the model are in agreement with earlier studies on factors predicting the need for surgery.[1,2,11] However, in contrast to other studies that have shown that assessment of PF variables such as total peritoneal fluid protein concentration and hemolysis may provide useful diagnostic information,[2,4,30] the present study did not detect any further advantages of adding PF variables to the clinical and blood variables. The reason for this finding probably relates to our inclusion criteria, in which only severe colic cases were included. Previous studies included less severe abdominal disease, such as simple large intestinal obstructions, when comparing medical and surgical abdominal conditions. Obstructions and other mild medical abdominal disease often cause little change 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 in which 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 were evaluated, since only a few horses requiring surgery had a second PF sample collected. Changes in PF lactate concentration have recently been reported to be valuable in identifying horses requiring surgery when serial

measures were performed.[31] 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 SAA concentration in serum was lower than with previous models.[1,2,30]

Serum Amyloid A concentration in serum has been measured in several studies investigating various inflammatory diseases in horses.[12] In this study, SAA concentration in serum was found to have significant diagnostic capacity in differentiating inflammatory from surgical colic. Including assessment of SAA concentration in serum 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 APP and lactate concentrations in horses with colic.[16] Adding pre-admission duration of colic to the model did not change the diagnostic performance of SAA concentration in serum or the model significantly. This is likely because SAA in serum is a fast-reacting biomarker that is significantly increased in horses with colic at durations of only 5 – 12 hours.[16] Plasma fibrinogen and serum Hp concentrations and WBC counts increase later and thus are more dependent on duration of disease.[16] When evaluated as single variables, plasma fibrinogen concentration and WBC count had significant diagnostic capacity, but when added to the clinical model they did not perform as well as SAA concentration in serum, despite taking duration of colic into consideration. This might be because of the large span of the duration intervals used in this investigation, or because of inaccurate estimations of colic duration given owners.

Because of the acute nature of equine colic and the need for rapid decision-making, a biomarker will only be useful diagnostically if the analysis can be performed on a single sample basis, within a short time, and at a reasonable price.[32] Such point of care tests are currently commercially available.[33]

In conclusion, considering SAA concentration in serum along with the clinical assessment of horses with severe colic improved the identification of horses with acute inflammatory colic that did not require surgery. Including SAA concentration in serum in the evaluation of horses with colic may minimize the risk of unnecessary or delayed surgery. Validation of the model on a different population of horses with severe colic showed that the model was valid in different hospital settings.

Footnotes

[a]Hawksley Medical and Laboratory Equipment, Lancing, UK

[b]Atago Sur-Ne Clinical Refractometer, ATAGO CO., LTD, Tokyo, Japan

[c]ADVIA 120, Bayer A/S, Lyngby, Denmark

[d]ADVIA 2120, Siemens Health Care Diagnostics Inc., NY

[e]Radiometer ABL725, Radiometer Medical ApS, Brønshøj, Denmark

[f]Accusport Blood Lactate Analyzer, Roche Diagnostics, Basel, Switzerland

[g]EIKEN Chemical Co. Ltd., Tokyo, Japan

[h]ADVIA 1800 Chemistry System, Siemens Health Care Diagnostics Inc., IL

[i]Tridelta Development Ltd., Ireland

[j]ACL 9000, Instrumentation Laboratory, Barcelona, Spain

[k]SAS, Institute SAS Inc., Cary, NC

References

1. Parry BW, Gay CC, Anderson GA. Assessment of the necessity for surgical intervention in cases of equine colic: a retrospective study. *Equine Vet. J* 1983;15(3):216–221.

2. Thoefner MB, Ersboll BK, Jansson N, et al. Diagnostic decision rule for support in clinical assessment of the need for surgical intervention in horses with acute abdominal pain. *Can J Vet Res* 2003;67(1):20–29.

3. Reeves MJ, Curtis CR, Salman MD, et al. Multivariable prediction model for the need for surgery in horses with colic. *Am J Vet Res* 1991;52(11):1903–1907.

4. Freden GO, Provost PJ, Rand WM. Reliability of using results of abdominal fluid analysis to determine treatment and predict lesion type and outcome for horses with colic: 218 cases (1991–1994). *J Am Vet Med Assoc* 1998;213(7):1012–1015.

5. Matthews S, Dart AJ, Reid SWJ, et al. Predictive values, sensitivity and specificity of abdominal fluid variables in determining the need for surgery in horses with an acute abdominal crisis. *Aust Vet J* 2002;80(3):132–136.

6. White NA, Elward A, Moga KS, et al. Use of web-based data collection to evaluate analgesic administration and the decision for surgery in horses with colic. *Equine Vet J* 2005;37(4):347–350.

7. Mair TS, Smith LJ, Sherlock CE. Evidence-based gastrointestinal surgery in horses. *Vet Clin North Am Equine Pract* 2007;23(2):267–292.

8. Feary DJ, Hassel DM. Enteritis and colitis in horses. *Vet Clin North Am Equine Pract* 2006;22(2):437–479.

9. Southwood LL. Acute abdomen. *Clin Tech Equine Pract* 2006;5(2):112–126.

10. Parry BW. Use of clinical pathology in evaluation of horses with colic. *Vet Clin North Am Equine Pract* 1987;3(3):529–542.

11. Johnston JK, Morris DD. Comparison of duodenitis proximal jejunitis and small intestinal obstruction in horses – 68 Cases (1977–1985). *J Am Vet Med Assoc* 1987;191(7):849–854.

12. Jacobsen S, Andersen PH. The acute phase protein serum amyloid A (SAA) as a marker of inflammation in horses. *Equine Vet Educ* 2007;19(1):38–46.

13. Vandenplas ML, Moore JN, Barton MH, et al. Concentrations of serum amyloid A and lipopolysaccharide-binding protein in horses with colic. *Am J Vet Res* 2005;66(9):1509–1516.

14. Forbes G, Sorich E, Nath LC, et al. Activin A as a novel biomarker of equine inflammatory abdominal disease: preliminary findings. *Vet J* 2011;190(2):154–156.

15. Prasse KW, Topper MJ, Moore JN, et al. Analysis of hemostasis in horses with colic. *J Am Vet Med Assoc* 1993;203(5):685–693.

16. Pihl TH, Scheepers E, Goddard A, et al. Influence of disease process and duration on acute phase proteins in serum and peritoneal fluid of horses with acute abdominal pain. *J Vet Intern Med* 2015; 29(2):651-658..

17. Underwood C, Southwood LL, McKeown KP, et al. Complications and survival associated with surgical compared with medical management of horses with duodenitis-proximal jejunitis. *Equine Vet J* 2008;40(4):373–378.

18. Cohen ND, Toby E, Roussel AJ, et al. Are feeding practices associated with duodenitis-proximal jejunitis? *Equine Vet J* 2006;38(6):526–531.

19. Harris N, Jou JM, Devoto G, et al. Performance evaluation of the ADVIA 2120 hematology analyzer: an international multicenter clinical trial. *Lab Hematol* 2005;11(1):62–70.

20. Schulman ML, Nurton JP, Guthrie AJ. Use of the Accusport semi-automated analyser to determine blood lactate as an aid in the clinical assessment of horses with colic. *J S Afr Vet Assoc* 2001;72(1):12–17.

21. Jacobsen S, Kjelgaard-Hansen M, Petersen HH, et al. Evaluation of a commercially available human serum amyloid A (SAA) turbidometric immunoassay for determination of equine SAA concentrations. *Vet J* 2006;172(2):315–319.

22. Pihl TH, Andersen PH, Kjelgaard-Hansen M, et al. Serum amyloid A and haptoglobin concentrations in serum and peritoneal fluid of healthy horses and horses with acute abdominal pain. *Vet Clin Pathol* 2013;42(2):177–183.

23. Andersen SA, Petersen HH, Ersbøll AK, et al. Vaccination elicits a prominent acute phase response in horses. *Vet J* 2012;191(2):199–202.

24. Altman, DG. (1991) Relation between several variables. In: Practical statistics for medical research, first edn., Chapman & Hall, Boca Raton. pp. 325–364.

25. Hosmer DW, Hosmer T, Le CS, et al. A comparison of goodness-of-fit tests for the logistic regression model. *Stat Med* 1997;16(9):965–980.

26. Wiinberg B, Jensen AL, Johansson PI, et al. Development of a model based scoring system for diagnosis of canine disseminated intravascular coagulation with independent assessment of sensitivity and specificity. *Vet J* 2010;185(3):292–298.

27. Bossuyt PM, Reitsma JB, Bruns DE, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Clin Chem* 2003;49(1):7–18.

28. Lemeshow S, Teres D, Pastides H, et al. A method for predicting survival and mortality of ICU patients using objectively derived weights. *Crit Care Med* 1985;13(7):519–525.

29. Reeves MJ, Curtis CR, Salman MD, et al. Validation of logistic-regression models used in the assessment of prognosis and the need for surgery in equine colic patients. *Prev Vet Med* 1992;13(3):155–172.

30. Ducharme NG, Pascoe PJ, Lumsden JH, et al. A computer-derived protocol to aid in selecting medical versus surgical treatment of horses with abdominal pain. *Equine Vet J* 1989;21(6):447–450.

31. Peloso JG, Cohen ND. Use of serial measurements of peritoneal fluid lactate concentration to identify strangulating intestinal lesions in referred horses with signs of colic. *J Am Vet Med Assoc* 2012;240(10):1208–1217.

32. Kjelgaard-Hansen M, Jacobsen S. Assay validation and diagnostic applications of major acute-phase protein testing in companion animals. *Clin Lab Med* 2011;31(1):51–70.

33. Jacobsen S, Kjelgaard-Hansen M. Evaluation of a commercially available apparatus for measuring the acute phase protein serum amyloid A in horses. *Vet Rec* 2008;163(11):327–330.