The acute-phase response is part of the innate immune response to inflammatory stimuli such as infection, trauma, and neoplasia. Human physicians use measurement of APP concentrations to rapidly detect and characterize inflammation. Acute-phase protein concentrations are used for diagnostic and prognostic purposes for diverse disease processes including cardiovascular disease, cancer, and sepsis. Abnormally increased serum concentrations of APPs have been reported in horses with colic and infectious diseases and following elective and nonelective surgeries. Acute-phase protein concentrations can either increase (positive APPs) or decrease (negative APPs) in response to inflammation. Positive APPs are categorized on the basis of their degree of response to inflammation. Plasma concentrations of major APPs increase >10-fold in response to inflammation, whereas moderate APPs increase 2- to 10-fold, and minor APPs increase <2-fold. Fibrinogen, a moderate APP, is the most commonly evaluated APP in equine medicine. However, it is a fairly insensitive marker of inflammation because of its wide reference interval, response time (24 to 72 hours following an inflammatory stimu-

**Evaluation of serum amyloid A and haptoglobin concentrations as prognostic indicators for horses with colic**

Trina L. Westerman DVM, MS
Crystal M. Foster MT
Susan J. Tornquist DVM, PhD
Keith P. Poulsen DVM, PhD

From the Departments of Clinical Sciences (Westerman, Poulsen) and Biomedical Sciences (Foster, Tornquist), College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331. Dr. Westerman’s present address is Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606. Dr. Poulsen’s present address is Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

Address correspondence to Dr. Westerman (tlwester@ncsu.edu).

**OBJECTIVE**
To evaluate the use of the acute-phase proteins serum amyloid A (SAA) and haptoglobin as prognostic indicators in horses with colic with regard to the need for surgical intervention, development of complications, and hospitalization cost and duration.

**DESIGN**
Prospective observational study.

**ANIMALS**
20 clinically normal horses and 42 horses with colic.

**PROCEDURES**
Total WBC and neutrophil counts and plasma fibrinogen, SAA, and haptoglobin concentrations were compared between healthy (control) horses and horses admitted to a veterinary teaching hospital for colic. Clinicopathologic values were compared between medical and surgical colic cases to test the ability of acute-phase proteins to predict indication for surgical intervention, development of complications, and duration and cost of hospitalization.

**RESULTS**
Mean SAA concentration was significantly higher in the surgical group, compared with that for both the control and medical groups. Haptoglobin concentration did not differ significantly among groups. Horses with colic and an abnormally increased SAA concentration (>5 μg/mL) were more likely to be managed surgically than medically (OR, 5.7; 95% confidence interval, 1.4 to 22.8). Horses with small intestinal lesions had significantly higher SAA concentrations than did control horses. Euthanasia due to a poor prognosis or the development of thrombophlebitis was more likely for horses with an SAA concentration >5 μg/mL (OR, 7.6; 95% confidence interval, 1.1 to 52.4). A weak positive correlation ($r=0.30$) was observed between cost of treatment and SAA concentration.

**CONCLUSIONS AND CLINICAL RELEVANCE**
Horses with colic that had an abnormally increased SAA concentration were more likely to require surgical intervention, develop thrombophlebitis, or be euthanized because of a poor prognosis despite treatment. ([J Am Vet Med Assoc 2016;248:935–940])

**ABBREVIATIONS**
APP: Acute-phase protein
IQR: Interquartile range
SAA: Serum amyloid A
lusion of leukocyte chemotaxis and activation of inflammatory mediator synthesis. The primary function of haptoglobin is to bind free hemoglobin to prevent iron loss.

Previous studies evaluating APPs in horses with signs of acute abdominal pain indicate that SAA concentrations in horses with inflammatory causes of gastrointestinal tract disease such as colitis, peritonitis, and proximal enteritis are increased, compared with those of horses with noninflammatory causes of colic. Evaluation of SAA concentration is also useful for the determination of horses with grass sickness from those with other noninflammatory causes of colic. In a prior study by Vandenplas et al, significantly more horses with colitis, enteritis, and peritonitis had an SAA concentration > 50 μg/mL, compared with horses with nonstrangulating and strangulating large and small intestinal obstructions. To our knowledge, studies evaluating SAA and haptoglobin concentrations as diagnostic indicators for medical or surgical intervention in horses with colic have not been conducted.

The objectives of the study reported here were to compare SAA and haptoglobin concentrations between clinically normal horses and horses with colic that were referred to a tertiary care facility and between horses with colic that underwent medical treatment and those for which surgical intervention was recommended, to evaluate the respective associations of SAA and haptoglobin concentration with duration of hospitalization, development of complications, death, and cost of treatment. The null hypotheses were that SAA and haptoglobin concentrations would not differ between horses with colic that received medical treatment and those for which surgery was recommended.

Materials and Methods

Animals

All study procedures were reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee. The study consisted of 3 (control, medical, and surgical) groups of horses. The control group consisted of clinically normal horses from the Oregon State University teaching herd or privately owned horses. The medical and surgical groups consisted of horses with signs of acute abdominal pain referred to the Oregon State University Large Animal Hospital. Horses with primary gastrointestinal inflammatory disease (peritonitis and colitis) or other systemic diseases that were not related to the primary gastrointestinal tract disorder were excluded from the study. The medical group included horses with nonstrangulating gastrointestinal tract disorders such as displacements, impactions, or gas colic that were treated medically. The surgical group included horses for which surgical intervention was recommended for correction of the underlying etiology. Owner consent was obtained prior to study enrollment for all privately owned horses.

Sample collection and processing

Blood (15 to 20 mL) was collected via jugular venipuncture into evacuated blood collection tubes that contained EDTA or sodium heparin, or tubes without an anticoagulant from horses with colic at the time of admission to the veterinary hospital as part of routine diagnostic testing during the initial examination. Blood (15 mL) was collected via jugular venipuncture into evacuated tubes that contained EDTA and tubes without an anticoagulant from control horses. Blood collection from control horses was performed in their home environments.

Blood samples collected during regular business hours were submitted to the Oregon State University Veterinary Diagnostic Laboratory for analysis, and blood samples collected outside of regular business hours were analyzed at the Oregon State University Veterinary Teaching Hospital. Blood samples collected into the tubes with sodium heparin were centrifuged at 2,200 g for 10 minutes to obtain plasma. A biochemical analysis was performed on each plasma sample by use of an automated analyzer at either the diagnostic laboratory or hospital. Plasma fibrinogen concentration was determined by the heat precipitation method. Plasma fibrinogen concentration is not typically performed during emergency hours; therefore, plasma fibrinogen concentration was not determined for horses admitted to the hospital outside of regular business hours. A CBC was performed on blood samples collected into tubes with EDTA by use of an automated analyzer at either the diagnostic laboratory or hospital. Blood samples collected into tubes without an anticoagulant were allowed to clot and then centrifuged at 3,000 g for 10 minutes to obtain serum. The serum was harvested from each sample, frozen, and stored at –80°C until batch analysis for SAA and haptoglobin concentrations.

An automated biochemical analyzer and commercially available assays were used to determine SAA and haptoglobin concentrations in all serum samples. Both assays had been validated for use in horses and were performed in accordance with the manufacturers' instructions and the standard operating procedure for new tests in the Oregon State University Veterinary Diagnostic Laboratory.

Data collection and analysis

Signalment was obtained for all study horses. Additional data collected from horses in the medical and surgical groups included diagnosis, affected portion of
the gastrointestinal tract (stomach [gastric impactions], small intestines [strangulating and nonstrangulating obstructions], large intestines [displacements, impactions, or gas colic], or unknown), type of intervention (medical or surgical), complications (thrombophlebitis, laminitis, or euthanasia owing to poor prognosis despite treatment), date and reason for euthanasia, duration of hospitalization, and treatment cost.

Reference intervals for biochemical tests at Oregon State University Veterinary Diagnostic Laboratory are determined by the mean ± 2 SD for a control population. The quantitation limit for the SAA assay was ≤ 5 μg/mL. All control horses had an SAA concentration < 5 μg/mL; therefore, an SAA concentration > 5 μg/mL was considered abnormally increased. The reference interval for haptoglobin concentration was determined to be 10 to 70 mg/dL. Haptoglobin concentrations > 70 mg/dL were considered abnormally increased.

The Shapiro-Wilk test was used to assess the respective data distributions for WBC count, neutrophil count, plasma fibrinogen concentration, and SAA and haptoglobin concentrations for normality within each study group. Data for the control group were normally distributed, and data for the medical and surgical groups were not normally distributed. The median and IQR (25th to 75th percentiles) were provided for all variables for continuity of data presentation.

The Kruskall-Wallis test followed by the Dunn test for multiple comparisons was used to compare hematologic and biochemical variables among the 3 study groups (control, medical, and surgical) and between the control group and horses with colic that were categorized on the basis of the affected portion of the gastrointestinal tract (stomach, small intestines, large intestines, or unknown). The Mann-Whitney U test was used to compare hematologic and biochemical variables between horses with colic that did and did not develop complications. Nonparametric Spearman correlation was used to assess the respective associations of WBC count, neutrophil count, and fibrinogen, SAA, and haptoglobin concentrations with the duration of hospitalization and cost of treatment. For analyses of complications and cost of treatment, only horses for which treatment was elected following a minimum diagnostic workup at hospital admission were included; horses euthanized because of financial constraints were excluded from those analyses. For the analysis for duration of hospitalization, only horses that survived to discharge from the hospital were included in the analysis; horses euthanized while hospitalized, regardless of the reason, were excluded from that analysis. Fisher exact tests were used to compare categorical variables among the 3 study groups. All analyses were performed by commercially available statistical software, and values of P < 0.05 were considered significant.

### Results

### Horses

The control group consisted of 20 clinically normal horses. The medical group consisted of 21 horses with colic associated with large intestinal lesions (n = 13), an unknown etiology (7), and gastric impaction (1). The surgical group consisted of 21 horses with colic associated with small intestinal lesions (n = 14), large intestinal lesions (5), and gastric impaction (2). Large intestinal lesions included large colon displacements and impactions. Small intestinal lesions were most frequently strangulating obstructions (n = 11); however, 3 horses (2 with eosinophilic enteritis and 1 with an ileal impaction) had nonstrangulating small intestinal obstructions. Of the 42 horses with colic, 28 (66.7%) survived and were discharged from the hospital, 10 (23.8%) were euthanized because of financial constraints, and 4 (9.5%) were euthanized because of a poor prognosis despite treatment. All 14 horses euthanized belonged to the surgical group.

### Hematologic and biochemical findings

Descriptive statistics for select hematologic and biochemical variables for each study group were summarized (Table 1). The median neutrophil counts for

### Table 1—Descriptive statistics for select hematologic and biochemical variables for clinically normal horses (control group; n = 20) and horses with colic that underwent medical treatment (medical group; 21) or for which surgery was recommended (surgical group; 21).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Medical group</th>
<th>Surgical group</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count (WBCs/μL)</td>
<td>6.690 (5.933–7.178)</td>
<td>7.800 (6.605–9.100)</td>
<td>7.490 (6.500–9.405)</td>
</tr>
<tr>
<td>Neutrophil count (neutrophils/μL)</td>
<td>3.365 (3.120–3.830)</td>
<td>5.500 (3.845–7.000)</td>
<td>6.142 (3.950–7.600)</td>
</tr>
<tr>
<td>Fibrinogen (μg/mL)†</td>
<td>200 (125–300)</td>
<td>300 (175–300)</td>
<td>200 (100–300)</td>
</tr>
<tr>
<td>SAA (μg/mL)‡</td>
<td>&lt; 5 to &lt; 5</td>
<td>&lt; 5 to &lt; 5</td>
<td>&lt; 5–172.2</td>
</tr>
<tr>
<td>Haptoglobin (mg/dL)§</td>
<td>42.8 (27.6–49.8)</td>
<td>30.7 (19.4–44.2)</td>
<td>28.1 (15.5–50.4)</td>
</tr>
</tbody>
</table>

†Within a variable, value differs significantly (P < 0.05) from that for the control group. ‡Fibrinogen concentration was determined for all horses in the control group and only 6 horses in the medical group and 7 horses in the surgical group. §IQR not reported because all values were < 5 μg/mL. §Within a variable, value differs significantly (P < 0.05) from that for the medical group.
the medical and surgical groups were significantly ($P < 0.01$) higher than the median neutrophil count for the control group. All horses in the control group had SAA concentrations below the quantitation limit for the assay ($< 5 \mu g/mL$). The median SAA concentration for the surgical group was significantly ($P < 0.01$) higher than that for the control and medical groups. The median serum haptoglobin concentration did not differ significantly among the 3 groups.

The frequency distributions of horses with select hematologic and biochemical abnormalities were summarized (Table 2). The proportion of horses with abnormal WBC counts, neutrophil counts, and haptoglobin concentrations did not vary significantly among the 3 groups. Horses with colic that had an abnormally increased SAA concentration ($> 5 \mu g/mL$) were more likely to belong to the surgical group (OR, 5.7; 95% confidence interval, 1.4 to 22.8) than were horses with an SAA concentration $< 5 \mu g/mL$.

The median SAA concentration for horses with small intestinal lesions was significantly ($P = 0.02$) greater than that of control horses. Serum amyloid A concentration was abnormally increased in 8 of 14 horses with small intestinal lesions, 3 of 18 horses with large intestinal lesions, 2 of 7 horses with colic of unknown etiology, and 1 of 3 horses with gastric impactions. The median SAA concentration was $32 \mu g/mL$ (IQR, $< 5$ to $105.6 \mu g/mL$) for horses with small intestinal lesions, $< 5 \mu g/mL$ (IQR, $< 5$ to $8.9 \mu g/mL$) for horses with large intestinal lesions, and $< 5 \mu g/mL$ (IQR, $< 5$ to $346.9 \mu g/mL$) for horses with colic of unknown etiology.

Thirty-two horses were included in the cost of treatment analysis. The cost of treatment was not significantly correlated with WBC count, neutrophil count, or serum haptoglobin concentration. There was a significant weak positive correlation ($r = 0.30$) between SAA concentration and cost of treatment. Plasma fibrinogen concentration was determined for 11 of the horses included in the cost of treatment analysis, and there was a significant weak negative correlation ($r = -0.56$) between fibrinogen concentration and cost of treatment. Twenty-eight horses were included in the duration of hospitalization analysis. None of the hematologic or biochemical variables assessed were significantly correlated with the duration of hospitalization.

Seven horses developed complications (thrombophlebitis, $n = 3$; euthanasia owing to poor prognosis despite treatment, 4). No horses in the study developed clinical signs of laminitis. Horses with complications were more likely to have an abnormally increased SAA concentration than were horses that did not develop complications (OR 7.6; 95% confidence interval, 1.1 to 52.4). There was no correlation between the development of complications and WBC count, neutrophil count, fibrinogen concentration, or haptoglobin concentration.

### Discussion

Results of the present study indicated that SAA concentration was the most sensitive variable assessed associated with the requirement for surgical intervention or development of complications in horses with signs of acute abdominal pain. The median SAA concentration at hospital admission for horses in the surgical group was significantly higher than that for horses in the medical group. More than half (13/21 [62%]) of the horses with colic that required surgical intervention had an SAA concentration $> 5 \mu g/mL$, compared with 4 of 21 (19%) horses with colic that were medically managed. Although an SAA concentration $> 5 \mu g/mL$ at hospital admission was associated with higher odds of surgical intervention, overlap of SAA concentrations between groups suggested that an increase in SAA concentration at admission alone did not differentiate horses that required surgery from those that could be medically managed. This difference should be emphasized given that horses with signs of acute abdominal pain that had peritonitis and colitis were excluded from the present study. In a study that involved horses with colic of any etiology, horses with inflammatory disorders such as peritonitis and colitis were more likely to have an abnormally increased SAA concentration than were horses with obstructive lesions. Horses with primary inflammatory disorders were excluded from the present study because clinical signs, physical examination, and diagnostic test findings (eg, diarrhea, fever, and neutropenia) often differentiate those

### Table 2—Number (percentage) of horses from Table 1 with select hematologic and biochemical abnormalities.

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Control group</th>
<th>Medical group</th>
<th>Surgical group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukopenia (&lt; 6,000 WBCs/μL)</td>
<td>5 (25)</td>
<td>4 (19)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Leukocytosis (&gt; 12,000 WBCs/μL)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Neutropenia (&lt; 2,000 neutrophils/μL)</td>
<td>2 (10)</td>
<td>2 (14)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Neutrophilia (&gt; 6,000 neutrophils/μL)</td>
<td>0 (0)</td>
<td>7 (33)</td>
<td>11 (52)</td>
</tr>
<tr>
<td>Hyperfibrinogenemia</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>(fibrinogen concentration $&gt; 400 mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormally increased SAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(SAA concentration $&gt; 5 \mu g/mL$)</td>
<td>0 (0)</td>
<td>4 (19)</td>
<td>13 (62)</td>
</tr>
<tr>
<td>Abnormally increased haptoglobin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(serum haptoglobin concentration $&gt; 70 mg/dL)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>4 (19)</td>
</tr>
</tbody>
</table>

See Table 1 for remainder of key.
horses from horses with strangulating or obstructive lesions that may require surgery.

In the present study, there was a significant association between abnormally increased SAA concentration and small intestinal obstructions, whereas in another study, an abnormally increased SAA concentration was not associated with either strangulating or nonstrangulating small intestinal lesions. For the horses of the present study, most of the small intestinal lesions were strangulating obstructions, which was likely a reflection of the population of horses with colic examined at our tertiary hospital. The duration of clinical signs prior to hospital admission may also have contributed to the high proportion (8/14) of horses with small intestinal lesions that had abnormally increased SAA concentrations. The Oregon State University Large Animal Hospital emergency caseload consists of a large proportion of horses trailered for long distances because of geography and the lack of other tertiary veterinary care facilities in the state. The duration of colic is positively associated with SAA concentration.5

Median serum haptoglobin concentration at hospital admission did not vary significantly among the 3 groups of horses in the present study, which suggested that haptoglobin concentration was not useful as a diagnostic or prognostic indicator for horses with signs of acute abdominal pain. Additionally, an abnormally increased haptoglobin concentration was not associated with the development of complications. Haptoglobin does not begin to increase until 12 to 24 hours after an inflammatory stimulus, whereas SAA can increase by >100-fold within 6 to 12 hours after an inflammatory stimulus, which may explain why an abnormally increased haptoglobin concentration was not detected in many of the horses with signs of acute pain in the present study. However, determination of haptoglobin concentration for horses with colic likely does have clinical value. A serum haptoglobin concentration increased from reference limits at hospital admission for a horse with colic may suggest that the horse has a chronic inflammatory disorder. Determination of the concentrations for at least 1 positive major APP and 1 positive moderate APP has been recommended for evaluation of animals with clinical signs of disease. Evaluation of both major and moderate APP concentrations allows for a more detailed assessment of the severity and duration of a disease process because major APPs increase and decrease rapidly owing to their fairly short half-life, whereas moderate APPs increase and decrease at a slower rate.

The low constitutive expression of SAA in combination with its rapid and marked increase (>10-fold) following an inflammatory stimulus makes SAA concentration an ideal marker for inflammation in horses. The SAA concentration was below the quantitation limit of the assay for all horses in the control group of the present study, which was similar to previously reported values in clinically normal horses. The veterinary literature commonly uses an SAA concentration of <20 μg/mL as the cutoff for clinically normal horses; however, in the present study, an SAA concentration <5 μg/mL was used as the reference cutoff on the basis of the standard operating procedure for new tests in the Oregon State University Veterinary Diagnostic Laboratory. The SAA assay used in the present study was the same as that used in other studies, and the results yielded by the assay have been consistent among those studies.

In the present study, haptoglobin concentration was measured by an immunoturbidimetric assay, whereas in other studies, haptoglobin concentration was measured by the use of colorimetric and hemoglobin binding capacity assays. The reference interval (10 to 70 mg/dL) used for haptoglobin concentration in the present study represented the mean ± 2 SD for the haptoglobin concentrations for the 20 horses in the control group. The upper limit of the reference interval used in the present study was substantially lower than that (200 mg/dL) reported for the colorimetric and hemoglobin binding capacity assays. This difference emphasized the importance of the use of laboratory-specific reference intervals for interpretation of assay results, particularly when results of assays with different methodologies are compared.

Although all horses in the control group of the present study were clinically normal, 5 horses were leukopenic and 2 horses were neutropenic. The reference intervals used to interpret the WBC and neutrophil counts were those established by the Oregon State University Veterinary Diagnostic Laboratory, which is accredited by the American Association of Veterinary Laboratory Diagnosticians. The reason for the aberrations in the WBC and neutrophil counts of those horses is unknown, and those 5 horses remained clinically normal for at least 3 months after blood sample collection.

A major limitation of the present study was the small sample size. In particular, the high proportion (10/42) of horses euthanized because of financial constraints limited the number of horses available for analysis of complications, cost of treatment, and duration of hospitalization. An effective survival analysis could not be performed for the horses in the medical and surgical groups because only 4 horses were euthanized for reasons other than financial constraints. Another limitation of the present study was that fibrinogen concentration was measured for only 13 of the 42 horses in the medical and surgical groups because fibrinogen concentration was not measured at admission for horses admitted outside of regular business hours. However, results of other studies indicate that horses with signs of acute abdominal pain generally do not have an abnormally increased plasma fibrinogen concentration; therefore, increasing the sample size of the present study likely would not have significantly altered our findings.

Results of the present study suggested that measurement of SAA concentration at hospital admission could be useful as a diagnostic and prognostic indicator for horses with colic. Horses with an abnormally increased SAA concentration at hospital admission were more likely to require surgical intervention, develop substantial complications such as thrombophlebitis, and be euthanized because of a poor prognosis despite treatment than were horses that did not have an abnormally increased SAA concentration at hospital admission.
Acknowledgments

Supported by the Oregon State University College of Veterinary Medicine Department of Clinical Sciences Resident Training Grants (Westerman) and Oregon State University New Faculty Funding (Poulsen).

This manuscript represents a portion of a thesis submitted by Dr. Westerman to the Oregon State University Department of Clinical Sciences as partial fulfillment of the requirements for a Master of Science degree.

Footnotes

a. AU480, Beckman Coulter Inc, Brea, Calif.
b. Element DC Veterinary Chemistry Analyzer, Heska, Loveland, Colo.
c. Advia 120 Hematology, Siemens Healthcare Global, Munich, Germany.
d. Hematru Veterinary Hematology Analyzer, Heska, Loveland, Colo.
e. LZ Test SAA, Eiken Chemical Co, Tokyo, Japan.
f. Haptoglobin assay OSR6165, Beckman Coulter Inc, Brea, Calif.
g. GraphPad Prism, version 6.0, GraphPad Software, San Diego, Calif.

References