Concentrations of serum amyloid A and lipopolysaccharide-binding protein in horses with colic

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Objective—To determine concentrations of 2 acute-phase proteins (serum amyloid A [SAA] and lipopolysaccharide-binding protein [LBP]) in serum samples obtained from horses with colic and identify relationships among these acute-phase proteins and clinical data.

Animals—765 horses with naturally developing gastrointestinal tract diseases characterized by colic (ie, clinical signs indicative of abdominal pain) and 79 healthy control horses; all horses were examined at 2 university teaching hospitals.

Procedure—Serum concentrations of SAA and LBP were determined by immunoturbidometric and dot-blot assays, respectively.

Results—SAA and LBP concentrations were determined for 718 and 765 horses with colic, respectively. Concentrations of SAA were significantly higher in nonsurvivors than in survivors, and horses with enteritis or colitis and conditions characterized by chronic inflammation (eg, abdominal abscesses, peritonitis, or rectal tears) had SAA concentrations significantly greater than those for horses with other conditions. Serum concentrations of LBP did not correlate with outcome, disease process, or portion of the gastrointestinal tract affected.

Conclusions and Clinical Relevance—Circulating concentrations of SAA were significantly higher at admission in horses with colic attributable to conditions having a primary inflammatory cause (eg, enteritis, colitis, peritonitis, or abdominal abscesses) and were higher in horses that failed to survive the episode of colic, compared with concentrations in horses that survived. Serum concentrations of LBP did not correlate with survival. Analysis of these findings suggests that evaluation of SAA concentrations may be of use in identifying horses with colic attributable to diseases that have inflammation as a primary component of pathogenesis. (*Am J Vet Res* 2005;66:1509–1516)

Inflammation causes changes in circulating concentrations of a specific class of serum proteins (ie, the acute-phase proteins), which are synthesized by hepatocytes.[1,2] Many acute-phase proteins, such as C-reactive protein and **serum amyloid A (SAA)**, are involved in a host's nonspecific defense against the initial insult. After experimental or natural infection or inflammation, concentrations of SAA typically increase within 1 to 3 days and may return to within reference ranges within 3 to 5 days.[3–5] There is convincing evidence that there is an acute-phase response in horses because increases in serum concentrations of SAA have been documented in horses with experimentally induced inflammation, horses with various naturally developing diseases, and neonatal foals with infectious diseases.[3,6] Analysis of results of clinical studies in neonatal foals suggests that evaluation of SAA concentrations may aid in the diagnosis of disease and monitoring of treatment in foals with conditions such as pneumonia.

Endotoxemia has been associated with colic in horses, with movement of **lipopolysaccharide (LPS)** into the circulation causing a proinflammatory response characterized by synthesis of inflammatory cytokines (eg, tumor necrosis factor), alterations in tissue perfusion, and fever. By use of the limulus amoebocyte lysate assay to detect LPS, several investigators have determined that as many as 45% of horses with colic examined at veterinary teaching hospitals are endotoxemic, that most endotoxemic horses have intestinal strangulation obstruction or severe inflammatory intestinal diseases, and that prognosis for survival is inversely correlated with the concentration of LPS in circulation.[7–12] Of potential importance in such horses is the finding documented in other species[4] that serum concentrations of **LPS-binding protein (LBP)**, an acute-phase response protein that plays a central role in the host's response to LPS,[13,14] increase dramatically as part of the acute-phase response. Because of the innate sensitivity of horses to LPS, increases in circulating concentrations of LBP may have important clinical effects.

The purposes of the study reported here were to determine serum concentrations of SAA and LBP in a large population of horses with gastrointestinal tract disease (colic) that were admitted to 2 veterinary teaching hospitals and determine whether relationships existed among the concentrations of these 2 proteins and clinical data (eg, age, sex, breed, severity of

Received July 28, 2004.
Accepted January 12, 2005.
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Supported by the Morris Animal Foundation, the Link Equine Research Endowment, and the White Fox Farm Research Fund.
The authors thank Shay Bush, Cristina Cameron, Kathy Daniel, Dr. Jennifer Mullin, Michael O'Conor, Katherine Parks, Kathryn Shelton, Clayton Leathers, Rocky Mason, Travis Tull, Jarred Williams, and Lauren Wise for technical assistance.
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disease, type of underlying disease, portion of the gastrointestinal tract involved, and outcome).

Materials and Methods

Sample population—Two populations of horses were used in the study. Healthy horses were owned by 2 universities, whereas horses with colic were client-owned animals examined at the veterinary medical teaching hospitals at those universities. This study was approved by the appropriate institutional review board of each of the 2 participating universities (ie, Animal Care and Use Committee of the University of Georgia and the Clinical Research Review Committee of Texas A&M University).

Healthy horses—Healthy adult horses comprised 32 horses owned by the University of Georgia and 47 horses owned by Texas A&M University. Horses at the University of Georgia were 14 Quarter Horses, 13 Thoroughbreds, 3 Warmbloods, and 2 Arabians. These horses ranged from 3 to 15 years of age (mean, 9 years of age). The 47 healthy horses owned by Texas A&M University were all Quarter Horse or Quarter Horse-type (eg, Paint) horses that ranged from 3 to 18 years of age (mean, 10 years of age).

Horses with colic—Horses (n = 765) were admitted to the large animal teaching hospitals at the 2 universities for evaluation of gastrointestinal tract disease (ie, colic). Horses with clinical signs of pain for which causes unrelated to the gastrointestinal tract or abdominal cavity were identified were excluded from the study.

Serum samples—Serum samples were obtained from 79 healthy horses. These samples were used to establish reference ranges for serum concentrations of SAA and LBP.

Serum samples also were collected from horses with colic at the time of admittance to the veterinary medical teaching hospitals. Serum samples were partitioned into labeled microcentrifuge tubes and stored at –70°C until assayed. Samples collected at Texas A&M University were shipped frozen on dry ice to the University of Georgia for analysis. Each serum sample was thawed once for assay. When a sufficient amount of serum was available, each sample was assayed to determine both SAA and LBP concentrations.

Clinical data for horses with colic—Clinical data were obtained from review of medical records. Demographic data were obtained for each horse, including location (ie, university), age, sex, and breed. Variables considered as outcomes of interest were outcome of hospitalization (hereafter referred to as outcome), affected portion of the gastrointestinal tract, disease process, and disease diagnosis. Outcome was categorized as survival (ie, discharged alive from the hospital) or nonsurvival. On the basis of written information included in the medical records, all horses that were euthanatized because of inoperable conditions, ruptured intestines, or other reasons for an extremely poor prognosis were categorized as nonsurvival; conversely, horses that were euthanatized because of financial reasons were excluded from the study. The affected portion of the gastrointestinal tract or abdominal cavity (stomach, small intestines, cecum, large colon, descending colon, peritoneum, liver, rectum, or unknown) and disease process (obstruction, strangulating obstruction, enteritis or colitis, peritonitis, intestinal perforation or rupture, ulcers, tympany, or unknown) were identified by review of the medical records.

Examination of information in the medical records was used to establish a diagnosis for the cause of colic for each horse. Diagnosis was also categorized as a **nonstrangulating large intestinal lesion (NLC)**, **strangulating large intestinal lesion (SLC)**, **nonstrangulating small intestinal lesion (NSI)**, **strangulating small intestinal lesion (SSI)**, other (peritonitis, colitis, rectal tears, intraabdominal abscesses, and other miscellaneous conditions), or unknown.

Data were recorded and initial decisions regarding classification of affected portion, disease process, and diagnosis were determined by veterinary students or technicians trained by the principal investigator at each of the universities. The decisions were subsequently reviewed by the principal investigator at each university. Data used to make each decision included information written in the medical record by the attending clinician and, when available, surgery or necropsy reports. For horses in which a disease category, affected portion, or disease process was not known, not reported, or not obvious, the horse was assigned to the appropriate unknown category.

Quantification of SAA—Serum concentrations of SAA were measured in duplicate by use of a modification of an automated immunoturbidometric assay described elsewhere.[4] The assay was performed on an analyzer[a] by use of polyclonal rabbit and monoclonal murine antibodies covalently bound to polystyrene latex particles.[b] Serum (3 µL) was mixed with 255 µL of HEPES buffer and then incubated with 75 µL of reagent containing the latex particles. The change in turbidity was measured at 660nM and adjusted by use of serial dilutions of a known SAA standard. Lowest limit of detection of the assay was 0.1 µg/mL. Coefficient of variation of this assay for samples with a concentration of 24 µg/mL (n = 30 samples) and 266 µg/mL (30 samples) was 6.5% and 2.7%, respectively.

Quantification of LBP—Serum concentrations of LBP were quantified with an immunoblot assay by use of goat anti-human LBP antibody[c] that recognizes equine LBP. Recombinant-human LBP was used as a standard. Initial immunoblots used to confirm specificity involved serum proteins resolved by the use of gel electrophoresis. Briefly, serum protein concentrations were determined by use of the Bradford method. Serum protein (20 µg) and known amounts of LBP standards were resolved by use of 10% SDS-PAGE and electroblotted onto nitrocellulose membranes. The membranes were blocked in buffer containing 7% nonfat milk, incubated with the primary antibody, washed to remove nonbound primary antibody, and incubated with the appropriate horseradish peroxidase–labeled secondary conjugate. Detection of antibody binding was performed by use of enhanced chemiluminescence detection kits[d] and autoradiography. The resultant autoradiograms were densitometrically scanned, and serum LBP was quantified by comparing the signal intensity to that of the standards. For dot-blot quantification, serum (20 µL) was diluted 1:5 in dilution buffer (20mM Tris-HCl [pH, 7.5], 1mM EDTA, 1mM ethyleneglycol-bis[β-aminoethylether]-tetraacetic acid, 1mM phenylmethylsufonyl fluoride, 5 mg of leupeptin/mL, and 5 µg of aprotinin/mL) and transferred by gravity onto nitrocellulose membranes through a 96-well dot-blot apparatus. Wells were washed twice with 200 µL of a solution containing 20mM Tris-HCl (pH, 7.5), 120mM NaCl, and 0.1% Tween-20, and transfer was confirmed by use of Ponceau S staining of the membranes.

Quantification of LBP was performed as described previously, relative to LBP standards on each membrane. The samples were divided into 11 groups, and mean LBP concentrations were determined from duplicate samples. Mean R^2 value for the 11 standard curves was 0.975. Lowest limit of detection of the assay was 0.3 µg/mL. Heavily lipemic samples (n = 8) did not transfer well to the membranes and were excluded from the study.

Data analysis—Samples with values less than the lower limit of detection of the assay (ie, 0.1 µg/mL for SAA and 0.3

μg/mL for LBP) were assigned a value of 0 for data analysis. Median and interquartile range were used to describe continuous data because these data appeared to have a non-Gaussian distribution. Categoric data were described by use of proportions and contingency tables. The relationship between dichotomous outcomes (eg, SAA concentration greater than the upper limit of the reference range) and independent categoric variables (eg, lesion type) was examined by use of 2-way and multiple-way contingency comparisons; the χ^2 and Fisher exact tests were used to compare proportions.[15,16] When results for testing of multiple-way contingency tables indicated a significant ($P < 0.05$) difference existed among groups, we collapsed categories of variables on the basis of the magnitude of the observed differences among groups. Because it can be argued that the probability of finding a significant result when testing only those larger differences identified after data were analyzed will be greater than that when test combinations are chosen at random before data analysis, the overall significance value for comparisons in the contingency table (ie, $P < 0.05$) was adjusted by dividing the significance value for any given comparison by the total number of pairwise comparisons in the contingency table in accordance with the method of Bonferroni. For example, to maintain an overall significance value of $P < 0.05$ for a contingency table with 8 categories of a dichotomous variable, we divided 0.05 by the number of possible pairwise comparisons in that table ([8!/2!] × [6 – 2]! = 28), such that significance for a given comparison from the table was 0.0018 (ie, 0.05/28). The relationship between the continuous outcomes (eg, concentration of SAA) and independent categoric variables (eg, anatomic site of the lesion) was examined by use of the Wilcoxon rank sum test (for dichotomous categoric variables) or Kruskal-Wallis test (for polytomous categoric variables).[9] Post hoc testing of Kruskal-Wallis results was performed by use of a modification of the method of Scheffé. Data analyses were conducted by use of commercial software.[e] A significance value of $P < 0.05$ was used for all analyses; methods for multiple comparisons in multiple-way contingency analyses or Kruskal-Wallis tests were adjusted as described to maintain an overall significance value of $P < 0.05$.

Results

Clinical population and outcome—Clinical data were obtained from 765 horses with colic from which sufficient serum was available to enable their inclusion in the study. Serum samples were tested for LBP concentrations in 765 horses and for SAA concentrations in 718 horses; there were 47 horses that had LBP concentrations determined but not SAA concentrations, and 8 horses that had SAA concentrations measured but not LBP concentrations. Four hundred seventy-eight (62%) horses were from the University of Georgia and 287 (38%) were from Texas A&M University. Sex of the horses included 337 (44%) castrated males, 105 (14%) sexually intact males, and 323 (42%) mares. Median age of the 765 horses for which sera were available was 8 years (range, 0.08 to 32 years; interquartile range, 4 to 14 years). Nineteen breeds of horses and 1 mule were represented; breed was not reported for 5 horses. The predominant breeds were Quarter Horses (n = 272; 36%), Quarter Horse-types (Paint [54; 7%] and Appaloosa [28; 4%]), Thoroughbreds (124; 16%), and Arabians (95; 12%). Survival status was recorded for all but 1 horse; 569 (74%) horses were discharged alive and 195 (26%) did not survive (died or were euthanatized).

Diagnosis—The diagnosis was categorized for all but 5 horses; 30 categories of diagnosis were reported. The most common diagnoses were colic of unknown cause (n = 200), large colon displacement (71), large colon impaction (61), colitis (51), small intestinal incarceration (46), ileal impaction (40), and large colon volvulus (35). Diagnosis was also categorized as NLC (270 horses), SLC (35), NSI (74), SSI (104), other (82), and unknown (200).

Affected portion—The most commonly affected portions of the gastrointestinal tract or abdominal cavity were the large colon (n = 283), small intestines (221), cecum (40), peritoneal cavity (ie, intraabdominal abscess or peritonitis; 40), descending colon (32), stomach (13), rectum (4), and liver (2). The affected portion was unknown for 130 (17%) horses.

Disease process—The disease process responsible for the condition was categorized. The disease processes included obstruction (n = 282), unknown (190), strangulating obstruction (129), enteritis or colitis (93), peritonitis (45), intestinal perforation or rupture (14), ulcers (8), and tympany (4).

SAA concentrations—Serum samples collected from 79 clinically healthy horses were used to determine a reference range. Median SAA concentration for these horses was 0 μg/mL, and the 97.5th percentile was 49.8 μg/mL. Mean ± SD concentration was 4.5 ± 11.4 μg/mL; thus, 2 SDs above the mean was 27.3 μg/mL. Given the skewed data, a percentile approach to the reference range was considered to be preferable.

Serum amyloid A concentrations were determined on samples obtained from 718 horses with colic at the time of admission to 2 veterinary medical teaching hospitals. Median concentration for all horses was 1.9 μg/mL, whereas the mean was 86 μg/mL. The interquartile range was 0.4 to 49.1 μg/mL and the range was 0 to 500.0 μg/mL. Because the data did not appear to have a Gaussian distribution, 2 approaches were used for analysis. First, nonparametric statistical methods were used for comparisons when the data were considered to be continuous. Second, the data were considered as a dichotomous variable (< 50 μg/mL or ≥ 50 μg/mL). We chose the concentration of 50 μg/mL because it was approximately the 3rd quartile for the horses with colic and the 97.5th percentile for the healthy horses (ie, upper limit of the reference range).

SAA concentration and outcome—Concentrations of SAA varied significantly for outcome ($P < 0.001$). Median values and interquartile ranges were less for survivors (median, 1.4 μg/mL; range, 0 to 500 μg/mL; interquartile range, 0.4 to 29.6 μg/mL), compared with values for nonsurvivors (median, 10.8 μg/mL; range, 0 to 500 μg/mL; interquartile range, 0.6 to 198.6 μg/mL). The proportion of nonsurvivors with SAA concentrations ≥ 50 μg/mL (36%; 180/645) was significantly ($P < 0.001$) greater than that of survivors (20%; 110/538).

SAA concentration and affected portion—Concentrations of SAA varied considerably within and among affected anatomic portions (**Table 1**). Values of

SAA were considerably higher for conditions involving the peritoneal cavity and rectum. Thirty-one of 38 (82%) horses with conditions involving the peritoneal cavity had SAA concentrations ≥ 50 µg/mL. The proportion of horses with conditions involving the peritoneal cavity or rectum with SAA concentrations ≥ 50 µg/mL (78%; 33/42) was significantly (P = 0.001) greater than that of horses with conditions affecting other portions of the gastrointestinal tract (22%; 147/676).

SAA concentration and disease process—Concentrations of SAA varied significantly among disease processes (**Table 2**). Concentrations were highest among horses with inflammatory lesions (enteritis or colitis; peritonitis). The proportion of horses with SAA concentrations ≥ 50 µg/mL was highest for horses with peritonitis and enteritis or colitis; the corresponding proportions were considerably lower for horses with other disease processes. The proportion of horses with enteritis or colitis or peritonitis that had SAA concentrations ≥ 50 µg/mL (63%; 79/126) was significantly (P = 0.002) greater than that of horses with all other disease processes (17%; 101/592).

SAA concentration and diagnosis—Concentrations of SAA varied among the 30 categories of diagnosis. When diagnosis was evaluated on the basis of category, SAA concentrations were significantly (P = 0.008) greater for horses with conditions categorized as other (ie, peritonitis, colitis, rectal tears, intraabdominal abscesses, and other miscellaneous conditions), whether compared as a dichotomous grouping or when compared by use of a multiple-grouping approach with post hoc testing (ie, other conditions vs NLC, SLC, SSI, NSI, and unknown; **Table 3**); no other diagnostic group differed significantly from the others. Concentrations of SAA were slightly higher in horses with conditions presumably associated with chronic, rather than acute, inflammatory conditions.

Serum LBP concentrations—Serum samples obtained from 79 healthy horses were used to determine the reference range. Median LBP concentration for these horses was 3.7 µg/mL, and the 97.5th percentile was 9.2 µg/mL. Mean ± SD concentration was 4.1 ± 2.3 µg/mL; thus, 2 SDs above the mean was 8.7 µg/mL. Although these data had a reasonably Gaussian distribution, a percentile approach was used for the reference range to be consistent with methods used for SAA concentrations and because 4 of the horses had values > 8.7 µg/mL (8.8, 9.1, 9.8, and 12.1 µg/mL, respectively).

Serum concentrations of LBP were determined for 765 horses with colic admitted to the 2 veterinary medical teaching hospitals. Median concentration for these horses was 7.1 µg/mL, whereas the mean was 14.0 µg/mL. The range was 0.2 to 235.7 µg/mL, and the interquartile range was from 2.7 to 17.6 µg/mL. Because the data for these horses did not appear to have a Gaussian distribution, 2 approaches were used for analysis. First, nonparametric statistical methods were used for comparisons when the data were considered to be continuous. Second, the data were considered as a dichotomous variable (< 9.2 µg/mL or ≥ 9.2 µg/mL). This value was chosen because 9.2 µg/mL represented the 97.5th percentile of the reference range.

LBP concentration and outcome—The LBP concentration did not vary significantly for outcome. There was no significant difference in LBP concentrations between survivors (median, 6.8 µg/mL; range, 0.2 to 235.7 µg/mL; interquartile range, 2.7 to 17.4 µg/mL) and nonsurvivors (median, 7.7 µg/mL; range, 0.2 to 86.7 µg/mL; interquartile range, 2.7 to 18.4 µg/mL). The proportion of survivors with LBP concentrations ≥ 9.2 µg/mL (42%; 238/569) was the same as that of nonsurvivors (42%; 81/195). However, 321 of 765 (42%) horses with colic had LBP concentrations that exceeded the upper limit of the reference range.

LBP concentration and affected portion—Concentrations of LBP did not vary substantially among affected anatomic portions, except for the liver. Concentrations of LBP were considerably higher for the 2 horses with liver disease (13.6 and 66.5 µg/mL, respectively); however, these data should be interpreted with caution because of the extremely small sample size. Median concentrations of LBP for all other anatomic sites ranged from 6.0 µg/mL for rectal disorders to 9.7 µg/mL for cecal disorders. Both horses with liver disease had an LBP concentration ≥ 9.2 µg/mL, whereas only 1 of 4 horses with rectal disorders had an LBP concentration ≥ 9.2 µg/mL. For the remaining portions of the gastrointestinal tract, the proportion of horses with LBP concentrations ≥ 9.2 µg/mL ranged

Table 1—Serum amyloid A (SAA) concentrations for the most commonly affected portions of the gastrointestinal tract or abdominal cavity in horses with colic.

Variable	Stomach (13)	Small intestine (204)	Cecum (38)	Large colon (265)	Descending colon (31)	Rectum (4)	Peritoneal cavity (38)	Liver (1)	Unknown (124)
Median (µg/mL)	3.2	1.6	1.2	1.1	26.6	257.4	500.0	8.8	1.7
Interquartile range (µg/mL)	0–15.5	0.3–21.8	0.3–9.7	0.4–52.3	3.3–90	11.0–500.0	68.6–500.0	NA	0.2–500.0
Proportion of horses with SAA concentration ≥ 50 µg/mL (%)	23	21	10	25	29	50*	82*	0	17

Numbers in parentheses indicate number of horses.
*Value differs significantly (P = 0.002) from value for other affected anatomic portions.
NA = Not applicable.

Table 2—Concentrations of SAA for the most common disease processes in horses with colic.

Variable	Obstruction (269)	Strangulating obstruction (118)	Enteritis or colitis (83)	Peritonitis (43)	Perforation or rupture (13)	Tympany (4)	Ulcers (8)	Unknown (180)
Median (μg/mL)	1.1	4.8	65.5	500	3.2	1.4	0.8	1.1
Interquartile range (μg/mL)	0.1–10.5	0.3–58.6	3.0–500.0	55.2–500.0	1.1–15.5	0–1.6	0–7.3	0.4–11.3
Proportion of horses with SAA concentration ≥ 50 μg/mL (%)	13	27	54*	79*	15	0	12	17

See Table 1 for key.

Table 3—Concentrations of SAA for the most common categories of diagnosis in horses with colic.

Variable	Nonstrangulating large intestine (253)	Strangulating large intestine (34)	Nonstrangulating small intestine (70)	Strangulating small intestine (94)	Other (78)	Unknown (189)
Median (μg/mL)	1.4	0.6	2.5	10.1	55.2	1.1
Interquartile range (μg/mL)	0.5–48.6	0–25.2	0.2–16.8	0.5–52.8	1.7–500.0	0.3–10.2
Proportion of horses with SAA concentration ≥ 50 μg/mL (%)	24	18	18	26	52*	17

See Table 1 for key.

from 40% (large colon [113/283] and small intestinal lesions [88/221]) to 52% (cecal disorders [21/40]).

LBP concentration and disease process—Serum LBP concentration did not vary by disease process. Median concentration of LBP was lowest for the 4 horses with tympany (2.8 μg/mL; interquartile range, 2.6 to 4.2 μg/mL) and highest for the 17 horses with intestinal rupture (median, 10.8 μg/mL; interquartile range, 3.8 to 17.7 μg/mL). The proportion of horses with an LBP concentration ≥ 9.2 μg/mL was greatest for horses with intestinal rupture (75% [6/8]) and lowest for horses with tympany (0% [0/4]) and ulcers (25% [2/8]). For all other categories of disease process, the proportion of horses with concentrations of LBP ≥ 9.2 μg/mL was between 38% (unknown cause [72/190]) and 47% (peritonitis [21/45]). Whether considered as a dichotomous or continuous variable, concentrations of LBP were similar for horses with strangulating obstruction and horses with obstruction or inflammatory lesions.

LBP concentration and diagnosis—Serum LBP concentration varied among the 30 categories of diagnosis. Mean values, interquartile ranges, and proportion of horses with an LBP concentration ≥ 9.2 μg/mL were highest for horses with abdominal abscesses, liver disease, intraabdominal adhesions, duodenitis or jejunitis of the proximal portion of the jejunum, intestinal rupture, cecal impaction, and neoplasia. Concentrations of LBP were similar for the diagnostic categories of other lesion (median, 9.2 μg/mL; range, 1 to 78.3 μg/mL), NLC (median, 7.2 μg/mL; range, 0.3 to 235.7 μg/mL), SLC (median, 4.3 μg/mL; range, 0.6 to 69.5 μg/mL), NSI (median, 8.0 μg/mL; range, 0.2 to 95.2 μg/mL), SSI (8.0 μg/mL; range, 0.4 to 68.4 μg/mL), and unknown (median, 5.8 μg/mL; range, 0.2 to 186.5 μg/mL). There were no significant differences in LBP concentrations among these categories of diagnosis.

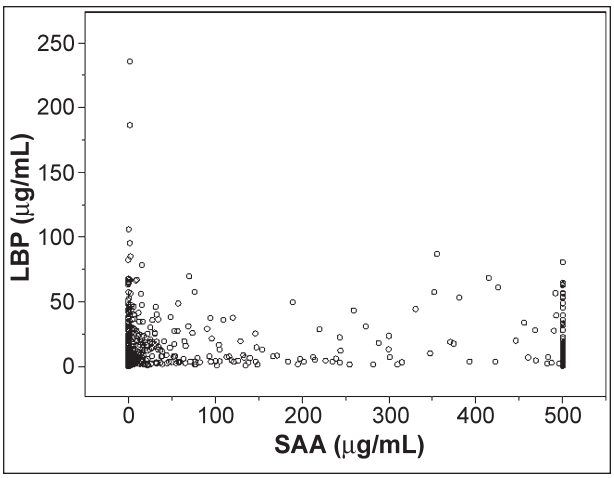

Figure 1—Concentrations of lipopolysaccharide-binding protein (LBP) and serum amyloid A (SAA) in serum samples obtained from 718 horses with colic examined at 2 veterinary medical teaching hospitals. Samples were obtained at the time of admission.

Association between concentrations of SAA and LBP—Concentrations of SAA and LBP were poorly correlated (**Figure 1**).

Discussion

Analysis of results of the study reported here indicates that determining the serum concentration of SAA but not LBP provides insight into the underlying disease process, anatomic region involved, and prognosis for horses examined because of signs of abdominal pain. Specifically, analysis of these results suggests that SAA concentrations ≥ 50 μg/mL are characteristic of horses with inflammatory diseases (enteritis or colitis; peritonitis) that have a poorer prognosis for survival than horses with other conditions.

Although several studies have been performed to determine the usefulness of other variables for prog-

nostic indicators in horses with colic, to our knowledge, this is the first large-scale study about the value of an acute-phase protein for identifying horses with conditions that have inflammation as a primary component of disease pathogenesis. These findings in adult horses with signs of abdominal pain are consistent with results that indicated neonatal foals with septicemia have significantly higher serum SAA concentrations than healthy foals[4] and that serum concentrations of SAA increase in horses with experimentally induced inflammation,[3,5,6] with naturally developing inflammatory diseases, undergoing elective surgical procedures,[6,17] and with experimentally induced infection with influenza virus.[18] It is important to mention that all of the serum samples analyzed in the study reported here were obtained at the time the horses were admitted to the veterinary medical teaching hospitals, and in many instances, the diagnosis was not known at the time these samples were obtained. Consequently, from a diagnostic standpoint, we are encouraged by the finding that a high proportion of horses with SAA concentrations ≥ 50 µg/mL had underlying inflammatory disease processes (enteritis or colitis; peritonitis) or specific diagnoses that involved inflammatory reactions (eg, abdominal abscesses). For many of these conditions, it is not easy to make a diagnosis, compared with making a diagnosis of acute intestinal obstruction or strangulating obstruction. Thus, increased serum concentrations of SAA in such horses may help increase a clinician's presumption that the underlying condition is a primary inflammatory problem or one in which inflammation is of primary importance to pathogenesis.

Although concentrations of SAA differed for some outcomes (ie, survivors vs nonsurvivors; horses with primary inflammatory conditions vs horses with other conditions), the sensitivity and specificity of SAA concentrations for a cutoff point of the upper limit of the reference range did not appear to be sufficiently high to be clinically useful. Many horses with inflammatory conditions and many horses that failed to survive had concentrations of SAA within the reference range, and many survivors had values that exceeded the reference range. Moreover, the predictive value of a positive result for the SAA concentration (ie, the probability that an SAA concentration that exceeded the reference range correctly classified a horse) will vary with the prevalence of the condition being evaluated. This study was conducted by use of horses at 2 veterinary medical teaching hospitals, and the predictive values of test results would likely differ when applied to horses examined by veterinarians in general practice or at other types of veterinary clinics.

The limited value of SAA concentrations for diagnostic testing is largely attributable to the variability in SAA concentrations among horses with similar clinical conditions, processes, or outcomes. Some of this variability may be attributable to the rate at which concentrations of SAA increase and then return to control values. Thus, the duration of the disease process prior to admission, the time at which samples were obtained for measurement of SAA concentrations in this study, likely influenced SAA concentrations in specific horses. Because the duration of the disease process could not be determined and may not always be accurately reflected by reported duration of clinical signs prior to admission, some of the variation in SAA concentration among horses may have been influenced by their stage of disease at the time of admission.

In contrast to our findings for SAA concentrations, evaluation of serum concentrations of LBP did not provide much insight into the pathogenesis or severity of the underlying conditions causing signs of abdominal pain in this large population of horses. Concentrations of LBP were not associated with outcome, affected anatomic portion, disease process, or diagnosis. Lipopolysaccharide-binding protein is an acute-phase protein that is important in mediating cellular responses to LPS. Studies[19,20] in other species indicate that the reference range of serum concentrations of LBP is 5 to 15 µg/mL and that serum LBP concentrations are increased in patients with gram-negative sepsis, systemic inflammatory response syndrome, and a combination of sepsis and septic shock. Results of 1 study[f] provide evidence that increased serum concentrations of LBP in patients with suspected gram-negative sepsis are associated with significantly higher mortality rates, suggesting an association between LBP concentration and severity of disease. Analysis of results of a clinical study[21] in which investigators compared LBP concentrations in people with infectious and noninfectious causes of systemic inflammatory response syndrome suggests that LBP is a nonspecific marker of the acute-phase response and cannot be used as a diagnostic tool for differentiating between these 2 processes.

We hypothesized that serum LBP concentrations would be increased in horses with diseases commonly characterized by clinical signs of endotoxemia and would correlate with a poor prognosis for survival. We based this hypothesis on the fact that endotoxemia is a potent stimulus for LBP synthesis in other species. However, other than intestinal rupture, serum concentrations of LBP were not increased in horses with disease processes commonly associated with the detection of endotoxin in circulation (eg, inflammatory lesions or intestinal ischemia), nor were serum concentrations associated with a poor prognosis for survival. Our finding that serum concentrations of LBP exceeded the upper limit of the reference range in 42% of horses with signs of abdominal pain and that LBP concentrations exceeded 9.2 µg/mL in 38% of horses with colic of unknown cause suggests that LBP also may be a nonspecific marker of the acute-phase response in horses. Because serum samples used in this study were obtained as part of the routine diagnostic testing for horses with signs of abdominal pain, it was not possible to measure endotoxin concentrations in these samples. Consequently, we were unable to assess any potential correlations between circulating concentrations of endotoxin and LBP.

We encountered a few difficulties in performing this large, multicenter clinical study that deserve specific mention. First, serum samples and clinical data were not obtained from each horse with signs of abdominal pain admitted to both veterinary medical teaching hospitals during the study period. Serum

samples were obtained only from those horses from which serum was obtained for diagnostic purposes and from which sufficient residual serum was available for testing and as a result of additional efforts made by the clinicians, veterinary students, and laboratory personnel at the 2 participating universities. Thus, although the findings of the study reported here may not reflect findings for all horses admitted during the study period, we are hopeful that having data from such a large population of affected horses from 2 study sites allowed us to make meaningful interpretations that apply to most horses with signs of abdominal pain.

Second, we found that a fairly large percentage of horses with signs of abdominal pain admitted at either veterinary medical teaching hospital had a diagnosis of colic of unknown cause or that the affected portion of the gastrointestinal tract was not definitively identified. Although these findings clearly reflect the reality of dealing with horses with signs of abdominal pain, it does make interpretation of increased serum concentrations of SAA or LBP in specific horses problematic. Because a large number of clinicians at the 2 institutions were involved in evaluation and treatment of the horses in this study, and because the financial constraints differed among clients, the extent of the diagnostic evaluation varied among horses.

Third, we encountered some difficulties with the medical records in our study, which is a situation that is common in retrospective clinical case studies. In an effort to minimize the effects such difficulties may have had on the conclusions reached in this study, 2 of the investigators (NDC and JNM) reviewed each medical record to determine a final decision about disease classification, affected portion, disease process, and outcome. Particular attention was paid to determine the reason a horse was euthanatized. Medical records indicating that a horse was euthanatized because of an inoperable condition, ruptured viscus, or obvious evidence of an extremely poor prognosis were included in the nonsurvival group. Conversely, horses with medical records containing information indicating that a horse was euthanatized because of financial reasons were excluded from the study. However, a small number of records were incomplete in this regard; therefore, the investigators decided on a case-by-case basis whether there was sufficient information to indicate that a horse had been euthanatized because of an extremely poor prognosis.

Several medical records (39/765 [5%]) listed > 1 diagnosis or disease process. These records were reviewed by the investigators to determine the primary diagnosis or disease process responsible for a horse's episode of colic at the time of admission. Although it is possible that serum concentrations of SAA or LBP may have reflected a secondary diagnosis or disease process, the secondary diagnosis or disease process in most horses developed or became evident days after the horses had been admitted to the veterinary medical teaching hospitals. Consequently, the investigators elected to focus on the clinical problem that most likely would have been of primary concern to a clinician evaluating the horse at the time of admission.

We are encouraged by our findings that concentrations of SAA ≥ 50 µg/mL at the time of admission to 2 veterinary medical teaching hospitals are characteristic of horses with underlying conditions that have inflammation as an important component of disease pathogenesis and a lower prognosis for survival than for horses with other conditions.In contrast, we were surprised to find that serum concentrations of LBP at the time of admission were of limited value. In consideration of these results, studies evaluating temporal changes in serum concentrations of these proteins in horses with colic may provide additional important information not identified in single samples obtained at the time of admission. Similarly, it would be interesting to monitor serum concentrations of these proteins after experimental administration of LPS to determine the effects of endotoxemia on concentrations of SAA and LBP in horses.

a. Hitachi 912, Hitachi High Technologies America, Schaumburg, Ill.
b. LZ test Eiken SAA Diagnostic Reagents, Eiken Chemical Co, Tokyo, Japan.
c. Provided by Dr. Peter S. Tobia, Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, Calif.
d. Enhanced chemiluminescence, Amersham Pharmacia Biotech, Piscataway, NJ.
e. S-PLUS, version 6.0, Insightful Inc, Seattle, Wash.
f. Carroll SF, Dedrick RL, White ML. Plasma levels of lipopolysaccharide binding protein (LBP) correlate with outcome in sepsis and other patients (abstr). *Shock* 1997;8:101.

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