Association between exercise-induced pulmonary hemorrhage and performance in Thoroughbred racehorses

Kenneth W. Hinchcliff, BVSc, PhD, DACVIM; Melissa A. Jackson, BSc; Paul S. Morley, DVM, PhD, DACVIM; James A. Brown, BVSc; Anthony F. Dredge, BVSc; Paul A. O’Callaghan, BVSc; John P. McCaffrey, BVSc; Ronald F. Slocombe, BVSc, PhD, DACVP; Andrew F. Clarke, BVSc, PhD

Objective—To determine whether exercise-induced pulmonary hemorrhage (EIPH) was associated with racing performance in Thoroughbred horses not medicated with furosemide and not using nasal dilator strips.

Design—Observational cross-sectional study.

Animals—744 two- to 10-year-old Thoroughbred horses racing in Melbourne, Australia.

Procedure—Horses were enrolled prior to racing, and a tracheobronchoscopic examination was performed after 1 race. Examinations were recorded on videotape, and presence and severity (grade 0 to 4) of EIPH were subsequently determined by 3 observers blind to the horses’ identity. Race records were abstracted for each horse examined.

Results—Overall, 52.1% of horses eligible for participation in the study were examined, and horses that were examined did not differ from horses that were not examined in regard to age, sex distribution, or proportion of horses that won or finished in the first 3 positions. Horses with EIPH grades ≤1 were 4.0 times as likely to win, 1.8 times as likely to finish in the first 3 positions, and 3.03 times as likely to be in the 90th percentile or higher for race earnings as were horses with grades ≥2. Horses with EIPH grades ≥1 finished significantly farther behind the winner than did horses without EIPH. However, odds that horses with grade 1 EIPH would win or finish in the first 3 positions were not significantly different from odds for horses without EIPH.

Conclusions and Clinical Relevance—Results suggest that EIPH is associated with impaired performance in Thoroughbred racehorses not medicated with furosemide and not using nasal dilator strips. (J Am Vet Med Assoc 2005;227:768–774)

From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210 (Hinchcliff); the School of Veterinary Science, University of Melbourne, Werr只知道, Victoria 3030, Australia (Jackson, Brown, Dredge, Slocombe, Clarke); the Animal Population Health Institute, College of Veterinary Medicine, Colorado State University, Fort Collins, CO 80523 (Morley); and Veterinary Services, Racing Victoria Ltd, 400 Epsom Rd, Flemington, Victoria 3031, Australia (O’Callaghan, McCaffrey).

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Address correspondence to Dr. Hinchcliff.

Exercise-induced pulmonary hemorrhage (EIPH) occurs commonly in Thoroughbred and Standardbred racehorses worldwide. Although estimates of the incidence of EIPH vary depending on the population of horses examined, the diagnostic method, and the frequency of examination, blood can be detected by means of tracheobronchoscopic examination of the airways in >50% of Thoroughbred horses after a race.1 2 The high incidence of EIPH has prompted speculation that EIPH is an important cause of impaired performance in Thoroughbred racehorses. Although this belief is strongly held by many horsemen and veterinarians involved in the care of racehorses, others have suggested that EIPH may be associated with superior performance, being reflective of greater racing effort, 3 4 and there currently is little scientific evidence to support either eventuality.

Previous studies 5 6 of Thoroughbred horses in which tracheobronchoscopic examination was performed after racing to detect EIPH have not found an association with performance. Studies7 8 of Standardbred racehorses have reported either no association between EIPH and performance, an association between EIPH and poor performance, or a tendency for EIPH to be associated with superior performance. However, the ability of previous studies to detect an association between EIPH and performance may have been impaired by inadequate statistical power, nonrandom selection of subjects, and administration of furosemide. Because races are won or lost by small margins, relative to the overall length of the race, examination of low numbers of horses would result in low statistical power and could prevent detection of an important effect of EIPH on performance. Furthermore, a large number of factors can affect the athletic performance of horses, and analysis of epidemiologic information on race performance requires appropriate sampling and use of sophisticated statistical analyses to account for collinearity among independent variables. Finally, although furosemide administration has been found to be associated with superior performance in racehorses, 7 8 its effect on the occurrence of EIPH has not been objectively demonstrated in racehorses.

On the basis of currently available evidence, it is unclear whether EIPH is associated with altered performance in racehorses. The purpose of the study reported here, therefore, was to determine whether there was an association between presence or severity of EIPH and race performance in Thoroughbred racehorses that were not medicated with furosemide and not using nasal dilator strips.

Materials and Methods

Experimental protocol—The study was designed as a cross-sectional study of a convenience sample of horses racing at racetracks in Melbourne, Australia, between March
and June 2003 whose owners and trainers agreed to participate. Thoroughbred racehorses were enrolled 24 to 48 hours prior to racing and were evaluated endoscopically after racing for evidence of EIPH. Data characterizing race performance of the horses were then analyzed to investigate potential associations with the occurrence of EIPH. Information regarding the horses' previous race performance was included in analyses to adjust for potential confounding.

Horses—Horses enrolled in the study were Thoroughbred racehorses of either sex competing in flat races at 1 of 4 racecourses in metropolitan Melbourne, Australia, between March 1 and June 18, 2003. All races were on turf and took place between 11:30 AM and 11:30 PM. Administration of medications, including furosemide, was not permitted on the day of the race, and this rule was stringently enforced by application of state-of-the-art drug testing procedures for detection of therapeutic and prohibited substances in blood and urine samples. It is therefore unlikely that horses in this study were administered agents that could have affected performance or development or severity of EIPH.

Control for any potential enrollment bias, horses included in the study were identified before racing. Prior to enrollment of any horses in the study, information regarding the study was distributed to trainers and owners of Thoroughbred racehorses by means of facsimile transmission to all registered trainers in the state, publication of articles in trade newsletters and newspapers, broadcast interviews on radio and television, live presentations at the racetracks, and personal contacts with influential trainers. Horses to be studied on a particular race day were identified 24 to 48 hours before the race. Lists of horses accepted to race were obtained from the body governing racing in this jurisdiction (Racing Victoria Ltd), and trainers of eligible horses were contacted by telephone to request permission to examine horses. The risks and benefits of the study were explained, and verbal permission to examine horses was obtained. On the day of the race, the study was again discussed with the trainers, horses were visually identified by one of the investigators, and written informed consent was obtained before the horse raced. After the race, horses were brought by their handlers to a central location at the racetrack and examined endoscopically within 2 hours after racing.

Detection and quantification of EIPH—All horses underwent tracheobronchoscopic examination for evidence of EIPH. Briefly, an endoscope was passed through 1 of the nares, and the nasopharynx, larynx, and trachea to the level of the carina were examined. Horses were not sedated for this procedure, and all examinations were recorded on videotape for subsequent analysis. Three individuals blinded to the identity of the horses and their race performance independently reviewed the videotapes and recorded their categorical assessments without discussing their observations with each other.

Severity of EIPH was graded on a scale from 0 to 4, with grade 0 indicating that no blood was detected in the pharynx, larynx, trachea, or mainstem bronchi visible from the tracheal bifurcation; grade 1 indicating the presence of 1 or more flecks of blood or ≤ 2 short (< 1/4 of the length of the trachea) streams of blood in the trachea or mainstem bronchi visible from the tracheal bifurcation; grade 2 indicating the presence of a long (> half the length of the trachea) stream of blood or > 2 short streams occupying less than a third of the tracheal circumference; grade 3 indicating multiple distinct streams of blood covering more than a third of the tracheal circumference but without evidence of blood pooling at the thoracic inlet; and grade 4 indicating multiple coalescing streams of blood covering > 90% of the tracheal surface with pooling of blood at the thoracic inlet.

Race records—Race records for horses included in the study were retrieved from a commercial database. Variables recorded on the day of the study, abstracted from the database, or obtained from the Bureau of Meteorology included horse name, age, sex, trainer, and jockey; race date; time of the race; racetrack, distance, and purse; weight carried; whether the horse finished the race; finishing position; finishing time of the winner; margin or distance finished behind the winner; speed rating for the race; number of horses in the race; days since last race; earnings for this race; lifetime earnings prior to this race; lifetime starts prior to this race; lifetime third-place finishes prior to this race; lifetime seconds-place finishes prior to this race; lifetime third-place finishes prior to this race; and presence of tracheal mucus. Investigators also recorded the time of each examination. Information obtained about the weather during the 24 hours preceding the race and at the time of the race included ambient temperature, humidity, rainfall, wind speed, and wind direction.

Data analysis—To determine whether EIPH was associated with race performance, distance finished behind the winner, race earnings, and finishing position were used as indicators of performance. Examination of summary and descriptive statistics and of graphs of the data was used to determine whether continuous data were normally distributed, and continuous data that were not normally distributed were transformed to yield a normal distribution or were categorized. The modal value of the EIPH severity grades assigned by the 3 observers was used in all analyses. Presence of EIPH was defined as a dichotomous (no vs yes) variable in 2 ways: severity grade of 0 (no) versus severity grade ≥ 1 (yes) and severity grade ≤ 1 (no) versus severity grade ≥ 1 (yes).

To control potential confounding, all variables that may have affected or predicted a horse’s performance were included as covariates in the analyses. However, a previous study has shown that there is considerable colinearity among these variables. Therefore, principal component analysis was used to create orthogonal (uncorrelated) scores for these independent covariates. Variables related to the race under investigation included in the principal component analysis were as follows: time between start of the race and endoscopic examination, weight carried, number of horses in the race, race distance, race purse, penetrometer reading, and horse age in years. Lifetime statistics included in the principal component analysis were as follows: lifetime starts prior to this race; lifetime earnings prior to this race; and days since last race and next-to-last race. Uncorrelated scores were calculated to create orthogonal (uncorrelated) scores for these independent covariates.

Different principal component scores were calculated for analysis of the relationship between EIPH and race performance and for the interaction between EIPH and race distance on performance.

With distance finished behind the winner and race earnings as dependent variables, potential associations with the occurrence of EIPH (EIPH severity grade; severity grade = 0 vs severity grade ≥ 1; and severity grade ≤ 1 vs severity grade ≥ 2) were examined by means of multivariable ANOVA. Because race earnings were highly skewed, they were log-normally transformed, with values of 0 assigned a nominal value of $1. Multivariate logistic regression was used to determine whether occurrence of EIPH was associated with various categorical assessments of finishing position and race earnings (ie, winning [yes vs no], finishing in the first 3 positions [yes vs no], earning money in the race [yes vs no], and being in the 90th percentile or higher for earnings in the race [yes vs no]). The Bonferroni method for multiple comparisons was used to adjust comparisons of least square means derived from ANOVA models. Odds ratios (ORs) and...
95% confidence intervals (CIs) derived from likelihood ratio statistics were calculated from the logistic regression models. Data are given as mean ± SE. For all analyses, values of \( P < 0.05 \) were considered significant.

**Results**

Tracheobronchoscopic examinations were performed on 744 horses competing in 202 races at 26 race meets. Horses were from the stables of 214 trainers, with no trainer contributing more than 41 horses (median, 2 horses; range, 1 to 41 horses) or 5.5% of the total number of horses examined. During the period of the study, there were 2,396 race starts by 1,428 horses in flat races at the race meets during which horses were examined. Mean ± SD number of horses in each race was 11.9 ± 2.5 horses. Overall, 52.1% of horses eligible for participation in the study were examined.

Horses examined ranged in age from 2 to 10 years (median, 4 years). The age distribution of horses that were examined was not significantly different from the age distribution of horses eligible for participation in the study that were not examined (Figure 1). Horses that were examined consisted of 306 females, 375 geldings, and 63 sexually intact males. Sex distribution for horses that were examined was not significantly different from sex distribution for horses eligible for participation in the study that were not examined (Figure 2). Of the 744 horses that were examined, 54 (7.3%) finished in first place and 170 (22.9%) finished in the first 3 positions.

Proportions of horses that were examined that won (\( P = 0.3 \)) or finished in the first 3 positions (\( P = 0.7 \)) were not significantly different from proportions of horses eligible for participation in the study that were not examined.

For the 744 horses that were examined, race distance ranged from 1,000 to 3,200 m. Mean ± SD time from the end of the race to the endoscopic examination was 31 ± 12 minutes, and blood was detected in the airways of 412 (55.3%) horses (Figure 3). Most of the horses with EIPH had only small amounts of blood in the airways (grade 1: 273/744 [36.7%]), and only 13 (1.7%) horses had grade 4 EIPH. Six horses had blood visible at 1 or both nostrils at the time of endoscopic examination. Five of these horses had grade 4 EIPH, and 1 had grade 2 EIPH.

Horses with EIPH severity grade ≤ 1 were 4.0 times as likely to win (95% CI, 1.5 to 14.3; \( P = 0.006 \)) and 1.8 times as likely to finish in the first 3 positions (95% CI, 1.05 to 3.07; \( P = 0.03 \)) as were horses with EIPH severity grade ≥ 2 (Figure 4). Horses with no evidence of EIPH (severity grade 0) were not significantly more likely to win (OR, 1.30; 95% CI, 0.68 to 2.47; \( P = 0.43 \)) or to finish in the first 3 positions than were horses with EIPH severity grade ≥ 1. Horses with grade 1 EIPH were no more likely to win than were horses with no evidence of EIPH (Figure 5).

Horses with EIPH severity grade ≥ 1 finished significantly (\( P = 0.002 \)) farther behind the winner (mean ± SE, 4.36 ± 1.16 m) than did horses with no evidence of EIPH (2.60 ± 1.07 m). For horses with EIPH, distance finished behind the winner was associated with grade of EIPH, with horses with higher grades finishing significantly (\( P = 0.025 \)) farther behind the winner (Figure 6). Post hoc testing indicated a significant dif-
ence in distance finished behind the winner for horses with grade 2 EIPH, compared with horses with no evidence of EIPH.

Earnings were also associated with EIPH severity grade. Horses with EIPH severity grade $\leq 1$ were 3.03 times as likely (95% CI, 1.33 to 7.96; $P = 0.002$) to be in the 90th percentile or higher for race earnings as were horses with EIPH severity grade $\geq 2$.

**Discussion**

Results of the present study corroborate findings of previous studies in which a high incidence of EIPH was found in Thoroughbred racehorses. Moreover, the present study revealed a consistent association, for Thoroughbred horses racing in Melbourne, Australia, between the presence of EIPH of severity grade $\geq 2$ and lower odds of winning or finishing in the first 3 positions, finishing a longer distance behind the winner, and a lower likelihood of being in the 90th percentile or higher for race earnings. We conclude, therefore, that EIPH is associated with impaired racing performance among Thoroughbred horses racing without treatment with furosemide or application of nasal dilator strips. Detection of an association between EIPH and performance in the present study does not prove causation. However, the high prevalence of EIPH severity grade $\geq 2$ (18.6%) in the present study and its association with measures of performance, combined with the well-documented effects of spontaneous or experimentally induced EIPH on lung function and arterial oxygen tension during exercise, suggest that EIPH is an important cause of impaired performance in Thoroughbred horses racing under the conditions of this study.

The association between EIPH and performance has been the subject of a number of studies. In those studies, a diagnosis of EIPH was made on the basis of detection of blood at the nostrils or detection of blood in the trachea or mainstem bronchi during endoscopic examination. However, use of epistaxis as the sole diagnostic criterion for EIPH is problematic, inasmuch as epistaxis is an insensitive indicator of EIPH (only 6/744 [0.8%] horses in the present study had epistaxis) and is usually present only in horses with severe EIPH (5/6 horses in the present study with epistaxis had grade 4 EIPH). Consequently, studies in which epistaxis is used as the sole criterion for diagnosing EIPH underestimate the incidence of EIPH and include almost exclusively those horses with the most severe form of the disorder. Such studies consistently demonstrate an association between epistaxis and impaired racing performance, as measured by the proportion of horses that win or finish in the first 3 positions. However, because EIPH was not detected in most affected horses, these studies do not provide information regarding the effect of lesser degrees of EIPH not associated with epistaxis. Studies relating epistaxis with racing performance therefore provide only limited information regarding the association of EIPH and performance.

Studies of Thoroughbred racehorses that have relied on tracheobronchoscopic examination for diagnosis of EIPH have, with 1 exception, not detected an
association between EIPH and performance. Possible reasons for this discrepancy between results of the present study and results of these previous studies include differences in study population, study design (including selection of horses), time between racing and endoscopic examination, sophistication of statistical analyses, and statistical power.

Reliance on tracheobronchoscopic examination for detection of EIPH presumes that blood will be present in the trachea or major bronchi at the time of examination in horses that have the disorder. Presumably, small quantities of hemorrhage in the peripheral regions of the lung may not be evident as blood in the trachea, and hemorrhage may not be detectable if insufficient time has elapsed between the occurrence of hemorrhage and tracheobronchoscopic examination for rostral movement of blood. Failure to detect minimal hemorrhage may not have adversely influenced results of the present study; however, as grade 1 EIPH was not associated with impaired performance. Furthermore, assuming that EIPH occurred during racing, the high tidal and minute volumes in horses during racing could be expected to propel blood rostrally, thereby minimizing the number of horses with false-negative tracheobronchoscopic results. These considerations highlight the inadequacy of our knowledge of the dynamics of pulmonary hemorrhage in horses during and after exercise. Although tracheobronchoscopic examination may have falsely ruled out the presence of hemorrhage in some horses, we believe that this would most likely have occurred only in horses with minimal hemorrhage and would not have affected the outcome of the present study.

Previous studies of the association between EIPH and performance in Thoroughbred horses have, with 1 exception, examined Thoroughbred horses racing in the United States, whereas the present study examined only horses racing in a single state in Australia. Geographic differences in airway health; racing and training conditions, including track surface; prevalence of other respiratory diseases, including inflammatory airway disease; training techniques; and medication use, among other factors, may have the potential to affect the predisposition to EIPH. Although associations between most of these variables and EIPH have not been demonstrated and we are not aware of studies documenting geographic differences in prevalence of EIPH, the possibility of regional differences should be considered when extrapolating results of the present study to conditions in other racing jurisdictions.

A previous study of Standardbred horses racing in New York detected a higher prevalence of EIPH, defined as blood covering more than half the tracheobronchial tree, in horses finishing seventh or eighth (33%) than in horses finishing first or second (21%). The authors of that study concluded that EIPH adversely affected performance, which is consistent with results of the present study. However, subsequent statistical analysis of the data led to the suggestion that EIPH may be associated with enhanced performance. These discrepant interpretations of the same data highlight shortcomings in the design and analysis of the study, including failure to control for variables that may impact performance, such as furosemide administration, use of a conservative definition of EIPH, and the lack of a grading system for severity of EIPH. The definition of EIPH in the study by MacNamara et al corresponds to grade 3 EIPH in the present study. Importantly, the previous study indicated that the proportion of horses finishing first or second that had chronic obstructive pulmonary disease (ie, excess tracheal mucus) was lower than the proportion of horses finishing seventh or eighth that had chronic obstructive pulmonary disease (10% and 39%, respectively), leading others to conclude that the presence of tracheal mucus is associated with poor performance. The prevalence of excessive tracheal mucus in the present study was low, and the presence of tracheal mucus was not associated with performance (data not shown).

Horses in the present study were not medicated with furosemide before racing, whereas horses in previous studies that did not detect an association between EIPH and performance were permitted use of furosemide on the day of racing. This could have affected results of these studies in 2 ways. First, furosemide administration is associated with better performance in Thoroughbred racehorses. Administration of furosemide to horses with EIPH may have attenuated or mitigated any detrimental effects of EIPH on performance, separate from any effect furosemide may have had on severity of EIPH, thereby preventing detection of an association between EIPH and impaired performance. Second, furosemide may have reduced the severity of EIPH, with a consequent improvement in performance. Currently, furosemide has not been demonstrated to reduce the severity of EIPH in horses under competitive racing conditions, although it does reduce the RBC count in bronchoalveolar lavage fluid collected after intense exercise on a treadmill. It is also conceivable that furosemide exerts concurrent and independent effects on performance and severity of EIPH. Horses in the present study had not been medicated with furosemide, which may have facilitated detection of an association between EIPH and performance that was not detectable in studies involving horses administered furosemide.

Previous studies have examined fewer horses than the present study, thereby reducing their ability to detect significant associations between EIPH and performance. Statistical power of the present study was enhanced by use of analytical techniques that account for the effects of confounding and colinearity among independent variables. Furthermore, previous studies examined only race placement as an indicator of performance, whereas the present study examined race placement, money earned, and distance finished behind the winner. The large number of horses in the present study, use of additional indicators of performance, and adjustment for confounding factors may have enabled us to detect an association between EIPH and performance that other studies were unable to detect.

Cross-sectional studies such as the present study and others that have investigated the association between EIPH and performance are susceptible to selection bias unless careful attention is paid to recruitment...
of subjects. Furthermore, the validity of the study is enhanced if the study sample is shown to be representative of the population from which it is drawn. Whereas previous studies have either not identified horses to be included in the study before racing, have examined horses on the basis of race placement, 7 or have not provided criteria used to include horses in the sample group, 1 the present study enrolled horses before racing, and the sample of horses that was examined was representative of the study population in regard to age and sex distributions and proportions of horses that won or finished in the first 3 positions. Furthermore, horses were examined only once, thereby preventing statistical and analytical problems associated with multiple examinations of the same horse. These efforts increase the likelihood that results of the present study can be extended to the larger population of Thoroughbred horses racing without medication with furosemide or use of nasal dilator strips. Whether the results of this study apply to horses of other breeds or horses racing after medication with furosemide or application of nasal dilator strips is unknown.

In the present study, an association between EIPH and performance was not detected when we defined presence of EIPH as horses with severity grade \( \geq 1 \) but was detected when we defined EIPH as horses with severity grade \( \geq 2 \). Our inability to detect an association between EIPH and performance when EIPH was defined as severity grade \( \geq 1 \) was attributable to the large number of horses with grade 1 EIPH and the lack of a detectable effect of grade 1 EIPH on performance. Furthermore, the fact that the association between EIPH and performance when EIPH was defined as severity grade \( \geq 2 \) was not attributable to the influence of a large number of horses with grade 3 or 4 EIPH indicates that EIPH of moderate severity is associated with impaired performance. The finding that grade 1 EIPH was not associated with impaired performance raises the question of whether minor hemorrhage has any clinical importance. Whether horses with grade 1 EIPH progress to having hemorrhage of greater severity is unknown.

On the basis of results of studies 9,10 of physiologic variables in horses with spontaneous or experimentally induced EIPH, it is plausible that there is an effect of EIPH on performance and that this effect may be related to the volume of blood in the airways. Arterial partial pressure of oxygen is lower during strenuous treadmill exercise in horses with EIPH than in unaffected horses, 9 and instillation of 200 mL of autologous blood into the airways of horses alters respiratory function at rest and reduces the maximal rate of oxygen consumption during exercise on a treadmill. 11,12 However, the effects of blood instillation on oxygen consumption and respiratory function appear to be related to the volume of blood instilled, with effects being detectable during exercise only after instillation of large volumes (200 mL) of blood. 4 This could explain our observation that there was no detectable association between grade 1 EIPH and performance in the present study, even though there was an association with EIPH severity grade \( \geq 2 \) and performance and an association between severity of EIPH and severity of impaired performance.

On the basis of results of the present study, we conclude that EIPH is associated with impaired performance by Thoroughbred horses racing in Australia without medication with furosemide or use of nasal dilator strips. The association between EIPH and impaired performance was apparent as a reduction in the likelihood that affected horses would win or finish in the first 3 positions, an increase in the distance that affected horses finished behind the winner, and a decreased likelihood that affected horses would have high winnings. Furthermore, there was an apparent dose-response relationship between severity of EIPH and severity of impaired performance, as evidenced by the association between severity of EIPH and distance the horse finished behind the winner.

References


a. Model CF-100TL (1.7 m in length and 1.1 cm in diameter) endoscope, Shikakawa Olympus Co Ltd, Tokyo, Japan.
b. i-RIS, Racing Victoria Ltd, Flemington, Victoria, Australia.
c. Bureau of Meteorology and Environmental Protection Authority, Melbourne, Victoria, Australia.
d. SAS, version 9.1, SAS Institute Inc, Cary, NC.


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**Selected abstract for JAVMA readers from the American Journal of Veterinary Research**

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

Michel L. Vandenplas et al

**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)