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# Evaluation of latex agglutination kits for detection of fibrin(ogen) degradation products and D-dimer in healthy horses and horses with severe colic

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**Background:** Fibrin(ogen) degradation products (FDPs) and D-dimer are sensitive indicators of excessive fibrinolysis due to disseminated intravascular coagulation (DIC) in dogs. To the authors' knowledge, latex-agglutination-based plasma FDP and D-dimer assays have not been validated for use in horses. **Objectives:** To determine: 1) sensitivity and specificity of latex-agglutination serum and plasma FDP and D-dimer assays for diagnosis of DIC; and 2) their prognostic value in horses with severe colic. **Methods:** At hospital admission and 24 hours later, blood was collected from 30 healthy horses and 20 horses with severe colic. Horses fulfilling predefined laboratory criteria of DIC were enrolled, and their data were subcategorized by survival for analysis. Platelet counts were determined and coagulation panel testing was performed. Serum and plasma FDP concentrations were measured using separate latex agglutination kits. Plasma D-dimer concentration was measured using 3 latex agglutination kits and a card immunofiltration test. Test sensitivity and specificity results were determined for healthy horses and those with colic. Median test values were compared between colic survivors and nonsurvivors to evaluate the prognostic usefulness of all tests. **Results:** Performance characteristics varied among assays and kit suppliers. The FDP assays had low sensitivity (<40%), whereas the most accurate D-dimer kit had 50% sensitivity and 97% specificity. High D-dimer concentration was the third most common hemostatic abnormality in horses with colic. Median antithrombin (AT) activity was significantly lower and activated partial thromboplastin time (aPTT) was significantly longer in nonsurvivors than survivors. **Conclusions:** Commercial latex-agglutination D-dimer assays might prove useful as adjunctive tests for the diagnosis of DIC in horses with severe colic; however FDP assays are invalid for this purpose. Low AT activity and prolonged aPTT at admission are associated with a poor prognosis in this patient population. (*Vet Clin Pathol.* 2005;34:375–382)

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**Key Words:** D-dimer, fibrin(ogen) degradation products, equine colic, disseminated intravascular coagulation

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Disseminated intravascular coagulation (DIC) is a common and potentially lethal complication of colic in horses. As many as 44% of horses with severe colic experience DIC.<sup>1–3</sup> Colic-initiated DIC is mediated by release of bacterial endotoxins that are absorbed through a devitalized, ischemic intestine. Endotoxemia induces cytokine release, which causes intravascular tissue factor expression. This results in widespread activation of the coagulation cascade, systemic generation of thrombin, and consumption of coagulation factors.<sup>4,5</sup> Endotoxins initially inhibit systemic fibrinolysis through upregulation of plasminogen activator inhibitor expression, and later promote fibrinolysis through activation of plasmin.<sup>4,6</sup> Horses with colic complicated by DIC are more likely to die or have extended hospital stays than are those without DIC.<sup>1,3,7,8</sup> Indeed, in a study of 37 horses with acute colitis, those with concurrent DIC were 8 times more likely to die.<sup>1</sup> Therefore, recognition of DIC is important because of its effect on patient care and outcome.

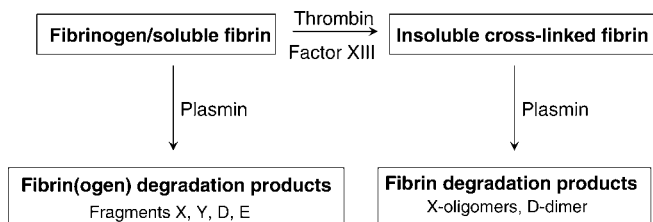
Diagnosis of DIC is based on a combination of clinical and laboratory findings. The criteria include a primary disease capable of initiating DIC, clinical signs of hemorrhage or thrombosis, and abnormal results of various laboratory tests of

hemostasis (ie, platelet count, coagulation assays, and assessment of inhibitors such as antithrombin [AT]) and fibrinolytic markers.<sup>5,9,10</sup> Although hemorrhage is usually recognized during clinical examination, thrombosis is often difficult to detect, and defining the extent of tissue damage caused by thrombosis requires extensive diagnostic evaluation. Horses generally develop thromboembolic, rather than hemorrhagic complications of DIC.<sup>1–3</sup> Diagnosis of DIC in horses more often is based on a combination of laboratory abnormalities, rather than on clinical signs of disease. Although DIC in horses is often considered “subclinical” because of lack of overt hemorrhage, the presence of metabolic abnormalities such as azotemia and high lactate concentration indicates that thrombosis has caused end-organ injury attributable to hypoxia.<sup>1</sup>

Systemic activation of thrombin and plasmin accompanies the extensive small vessel thrombosis of DIC. Several laboratory tests for detecting thrombin/plasmin activation in human patients are available; however, only a few tests have been validated for use in horses, including measurement of thrombin-AT complexes, fibrinogen degradation products (FDPs) and D-dimer.<sup>1,2,7,8,11,12</sup> D-Dimer is a degradation

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**Figure 1.** Schematic illustration of fibrin(ogen) degradation product (FDP) and D-dimer formation. Plasmin cleaves fibrinogen or soluble fibrin to yield FDPs. Soluble fibrin is converted to insoluble or cross-linked fibrin by factor XIIIa, which is activated by thrombin. Plasmin cleaves insoluble fibrin into cross-linked FDPs of variable molecular weight, called X-oligomers. D-Dimer is a neoepitope produced by cross-linking and is located within these X-oligomers.

fragment released exclusively by plasmin-mediated lysis of cross-linked fibrin. It is formed only after the sequential action of thrombin, then plasmin on a fibrinogen substrate. Thus, D-dimer is a specific indicator of fibrinolysis, in contrast to FDPs, which are fragments released by plasmin degradation of either fibrinogen or fibrin (Figure 1). D-Dimer has been used as a marker of fibrinolysis in horses that are competing in endurance races,<sup>13</sup> and in those with acute laminitis<sup>11</sup> and colic.<sup>2,7,12,14,15</sup>

Commercially available FDP and D-dimer assays are configured by use of antibodies raised against human FDPs.<sup>16</sup> Fibrin(ogen) degradation products in equine serum traditionally have been measured using latex-agglutination kits that contain polyclonal antibodies<sup>1,3,8</sup>; however, new monoclonal antibody-based assays for detecting FDPs in plasma are available and have been validated for use in dogs.<sup>17</sup> D-Dimer has been detected in horses by use of ELISA and card immunofiltration (IF) and automated immunoturbidometric assays.<sup>2,7,11–15</sup> Also, a latex-agglutination D-dimer kit was used recently to detect this fragment in neonatal foals.<sup>18</sup> To our knowledge, there have been no reported studies testing the ability of commercially available latex-agglutination FDP and D-dimer kits to detect fibrin and FDPs in plasma of horses with colic. Latex-agglutination assays have several advantages over ELISA and IF and immunoturbidometric assays, including their wider availability, ease of use, potential use as point-of-care tests, and rapid results.

The objectives of the study reported here were: 1) to determine the sensitivity and specificity of serum FDP, plasma FDP, and four D-dimer assays for the diagnosis of DIC in horses with severe colic, and to determine whether coagulation, FDP, or D-dimer assays are useful prognostic indicators in horses with severe colic and DIC. We hypothesized that, at the time of admission, detection of D-dimer would be a sensitive and specific indication of DIC in horses with colic and that high D-dimer values would be a negative prognostic indicator.

**Materials and Methods**

**Animals and sample collection**

Two groups of horses were evaluated: 1) clinically healthy horses (n = 30) and 2) horses with severe colic (n = 20),

which were presented to the Cornell University Hospital for Animals (n = 8) or the large animal clinic at Oregon State University (n = 12). Severe colic was defined clinically as tachycardia (>60 beats/minute), injected sclera, prolonged capillary refill time (>2 seconds), and abdominal pain that was poorly or not responsive to analgesics. The horses with colic were subgrouped as survivors and nonsurvivors.

Blood samples were collected via jugular venipuncture into Reptilase<sup>®</sup> (*Bothrops atrox* venom)-based clot tubes (Murex Diagnostics, Narcross, GA) for serum FDP analysis and into 3.2% citrate-containing tubes (1 part citrate to 9 parts blood) (Vacutainer, Becton-Dickinson Co, Franklin Lakes, NJ) for coagulation, plasma FDP, and D-dimer assays. An additional EDTA-anticoagulated sample for determining platelet counts was collected from horses with colic. Samples were collected at the time of admission (time 0) and 24 hours later (time 24) from horses with colic, and once from healthy horses. Client consent was obtained before blood withdrawal from client-owned animals. Only colic-affected horses that fulfilled predefined laboratory criteria of DIC within the first 24 hours (time 0 and/or 24) were included in the analyses. Criteria of DIC were 2 or more of the following abnormalities (compared with the laboratory’s reference intervals): thrombocytopenia, prolonged activated partial thromboplastin time (aPTT), prolonged prothrombin time (PT), hypofibrinogenemia, and low AT activity. Healthy horses were maintained at the Equine Research Park at Cornell University. The study was approved by the Institutional Animal Care and Use Committee at Cornell University.

**FDP and D-dimer assays**

Platelet-poor plasma was obtained immediately after collection of citrate-anticoagulated blood by centrifugation of the sample at 1000g for 10 minutes. Similarly, serum was obtained by centrifugation of the FDP clot tube after incubation at room temperature (approximately 24°C) for 30 minutes. All samples were stored at –20°C until they were analyzed in batches (within 1 month of collection). All assays were performed according to the manufacturers’ recommendations and included positive and negative controls.

Serum (Thrombo-Wellcotest, Murex Diagnostics, Narcross, GA) and plasma (FDP Plasma, Diagnostica Stago, Parsippany, NJ) FDP concentrations were determined using commercially available latex-agglutination kits. The serum FDP assay contains polyclonal antibodies against human FDPs, particularly fragments D and E<sup>19</sup>; the plasma FDP assay contains a monoclonal antibody against human FDPs. Serum samples were diluted 1:5 and 1:20, and plasma samples were diluted 1:2 and 1:8. Results were reported as negative (no agglutination, ie, <10 µg/mL of serum and <5 µg/mL of plasma) or positive (agglutination at either or both dilutions, ie, >10 µg/mL of serum and >5 µg/mL of plasma).

Plasma D-dimer concentration was measured using 3 latex agglutination kits: A (Accuclot D-dimer, Trinity Biotech, Wicklow, Ireland), B (D-Di Test, Diagnostica Stago), and C (Murex D-dimer, Murex Diagnostics), and 1 card IF kit, D (Nycocard, Nycomed Pharma AS Diagnostics, Oslo,

Norway). Each kit contains different monoclonal antibodies against human D-dimer. For all latex-agglutination assays, undiluted plasma samples were tested initially; undiluted samples with positive agglutination results then were serially diluted according to manufacturers' recommendations to provide semiquantitative results. Results were not directly comparable among kits due to differing analytic sensitivities of kit antibodies to D-dimer. Using kit D, D-dimer in the plasma sample is drawn through and binds to an antibody-impregnated membrane by filter action. A secondary colored colloidal gold antibody detects bound D-dimer, with the resulting color change being read visually to provide semiquantitative results, using a scale provided as a reference. Because healthy horses have D-dimer concentrations <1000 ng/mL (as determined by ELISA<sup>2,11</sup>), we chose  $\geq 1000$  ng/mL as a cut-off concentration between negative and positive D-dimer results. All possible cut-off concentrations also were displayed in receiver-operator characteristic (ROC) curves for the most accurate D-dimer kits (based on sensitivity and specificity). For kit A, results were reported as negative (<250, 250–500, 500–1000 ng/mL) or positive (1000–2000, >2000 ng/mL). For kit B, results were reported as negative (<500, 500–1000 ng/mL) or positive (1000–2000, >2000 ng/mL). For kit C, results were reported as negative (<1000 ng/mL) or positive (1000–2000, >2000 ng/mL). For kit D, results were reported as negative (<300, 300, 300–1000 ng/mL) or positive (1000, 2000, >2000 ng/mL).

#### Hemostasis assays (coagulation assays and platelet counts)

Activated partial thromboplastin time, PT, fibrinogen concentration, and AT activity were measured in citrated plasma samples using an automated coagulation analyzer (STACompact, Diagnostica Stago) and commercial reagents. The activation time for the aPTT (Dade Actin FS, Baxter Diagnostics, Edison, NJ) was 180 seconds. The PT reagent (Thromboscreen, Pacific Hemostasis, Huntersville, NC) used initially to analyze samples from all healthy horses and 8 colic-affected horses was discontinued by the manufacturer and was replaced with a substitute PT reagent (Thromboplastin LI, Helena Diagnostics, Beaumont, TX) for the remainder of the study (11 horses with colic, including 8 survivors and 3 nonsurvivors).

Fibrinogen concentration was assayed using the Clauss method<sup>20</sup> with a human thrombin reagent (100 NIH U/mL, Diagnostica Stago). A standard curve was derived from dilutions of a pooled equine plasma sample (prepared from 10 healthy horses) containing 300 mg of fibrinogen/dL. The fibrinogen content of the equine plasma standard was determined by use of a gravimetric assay of thrombin-clottable fibrinogen.<sup>21</sup>

Plasma AT activity was measured using a synthetic chromogenic substrate kit (Stachrom AT III, Diagnostica Stago) according to the manufacturer's recommendations, with the exception that the standard curve was derived from dilutions of pooled equine plasma rather than by use of a human plasma standard. Antithrombin activity in the study samples was reported as a percentage of the equine standard,

which had an assigned value of 100%. Reference intervals for the coagulation assays were defined as  $\pm 2$  SD from the mean on the basis of results of analysis of 12 (coagulation panel) or 20 adult horses (AT activity). The healthy study horses were not included in these determinations.

Platelet count was determined immediately after collection of EDTA-anticoagulated blood using automated hematology analyzers. An ADVIA 120 (Bayer Diagnostics, Tarrytown, NY) was used at Cornell University; a CELL-DYN 3500 (Abbott Laboratories, Abbott Park, IL) was used at Oregon State University. Wright's-stained blood smears were examined for platelet clumping (which falsely decreases the numerical count) and to estimate the platelet count (where 1 platelet =  $15 \times 10^9$ /L per  $\times 1000$  oil immersion field). The latter was performed to verify the numerical count from the analyzer and/or provide an estimated count (low, adequate, or high) when numerical counts were not obtained or were considered inaccurate (usually due to platelet clumping).

#### Statistical analyses

Because the FDP and D-dimer kits had differing sensitivities and cut-off concentrations, assay results were converted to an ordinal scale (in ascending order according to increasing concentration) for statistical evaluation. Only time-0 results from colic-affected horses were included in the analysis of sensitivity, specificity, and prognostic value.

Point estimates with 95% confidence intervals (CI) were calculated (Epi-info version 6.04b, 1997, Centers for Disease Control and Prevention, Atlanta, GA) for sensitivity and specificity of the FDP and D-dimer assays for diagnosis of DIC in horses. Sensitivity was defined as the proportion of colic-affected horses having positive FDP or D-dimer results (ie, D-dimer values  $\geq 1000$  ng/mL) at time 0. Specificity was defined as the proportion of healthy horses having negative results. Spearman rank correlations ( $R_{sp}$ ) were calculated (Statistix 8, 2003, Analytical Software, Tallahassee, FL) to determine associations at time 0 among coagulation assay, and FDP and D-dimer results in colic-affected horses. This was not done for the PT results, because the change in reagent resulted in longer clotting times in healthy horses.

To determine whether any test results at the time of admission were associated with outcome (survival vs death), median results were compared among groups (healthy, surviving, and nonsurviving horses) by use of a Kruskal-Wallis one-way ANOVA (Statistix 8). This was not done for the PT results, because of the change in reagent. Instead, the proportions of horses with PTs higher than the reference intervals (for both reagents) were compared using a Fisher exact test (Statistix 8).

Kappa statistics were calculated (Epi-info version 6.04b) to test concordance among pairs of FDP and D-dimer tests for positive and negative results. Kappa statistic <0.4 was considered poor and >0.75 was considered good to excellent.

To determine whether the coagulation assay, and FDP and D-dimer results in surviving horses with colic worsened with time, their results from times 0 and 24 were compared

**Table 1.** Median (range) of hemostasis test results in healthy and colic-affected horses at the time of admission. Colic-affected horses were classified as survivors or nonsurvivors.\*

Analyte	Healthy (n=30)	Survivors (n=11)	Nonsurvivors (n=9)	Reference Interval
aPTT (s)	51 <sup>a</sup> (41–63)	55 <sup>a</sup> (41–78)	76 <sup>b</sup> (50–81)	40–63
PT (s)	9.4 <sup>†</sup> (8.6–9.8)	10.3 <sup>†</sup> (9.9–11.4) 17.0 <sup>‡</sup> (10.7–23.6)	11.5 <sup>†</sup> (9.7–13.0) 18.6 <sup>‡</sup> (17.7–21.9)	8.0–11.0 <sup>†</sup> 13.5–18.5 <sup>‡</sup>
Fibrinogen (mg/dL)	329 <sup>a</sup> (218–622)	236 <sup>a</sup> (148–384)	300 <sup>a</sup> (127–780)	178–445
AT activity (%)	94 <sup>a</sup> (74–118)	85 <sup>a</sup> (66–122)	61 <sup>b</sup> (41–86)	86–130
Platelet count ( $\times 10^9/L$ )	ND	176 <sup>a</sup> (105–418)	150 <sup>a</sup> (100–354)	98–246

\*aPTT indicates activated partial thromboplastin time; PT, prothrombin time; AT, antithrombin; ND, not done. Medians with common superscripts are not significantly different ( $P \geq .05$ ). Comparisons were not done for the PT assay due to a change in reagent.

<sup>†</sup>PT assay was performed using initial reagent for samples from healthy horses (n=30), survivors (n=3), and nonsurvivors (n=5).

<sup>‡</sup>PT assay was performed using substitute reagent for samples from survivors (n=8) and nonsurvivors (n=4).

using a Wilcoxon signed-rank test (Statistix 8). For the PT results, paired data for prolonged PT at times 0 and 24 were tested for systematic change by use of McNemar's  $\chi^2$ -test (Statistix 8). For all analyses,  $P \leq .05$  (2-sided) was considered significant.

**Results**

Age of the 30 healthy horses ranged from 1 to 21 years (median, 9.5 years), with 21 mares and 9 geldings. Breeds included Warmblood (n = 11), Thoroughbred (8), mixed breed (6), Quarterhorse (2), Haflinger (1), Arabian (1), and Appaloosa (1). Blood samples were obtained from 22 horses with severe colic; however, 2 did not fulfill our predefined criteria for DIC at time 0 or 24 and were excluded from further analysis. Age of colic-affected horses ranged from 0.5 to 27 years (median, 6.5 years), with 11 mares, 8 geldings, and 1 stallion. Breeds included Thoroughbred (4), Quarterhorse (4), Warmblood (2), Belgian (2), Percheron (2), Arabian (2), Haflinger (1), Appaloosa (1), American Saddlebred (1) and Welsh pony (1). Of these 20 colic-affected horses, 11 survived the colic episode and were discharged, whereas 9 died or were euthanized within the first 24 hours of admission and had only a time-0 sample collected. Of the 11 surviving horses, 3 had small intestinal disorders, including 2 with incarceration (through the epiploic foramen or a hole in the mesentery) and 1 with strangulating lipoma, whereas 8 had large intestinal disorders, including 3 with impaction, 2 with colitis, and 1 each with strangulating lipoma, colonic torsion, and colonic displacement.

Within the first 24 hours of admission, 4 were treated medically and 7 were treated surgically. Of the 9 nonsurvivors, 4 had small intestinal disorders, including strangulating obstruction (2), strangulating lipoma (1), and ruptured abscess (1), whereas 5 had large intestinal disorders including 1 each of colonic impaction, colonic torsion, strangulating lipoma of the small colon, and ruptured colon secondary to rectal polyp or enterolith. Four were taken to surgery then euthanized due to severe, devitalized, or nonresectable lesions. On the basis of severity of clinical signs of disease and poor prognosis, the remaining 5 horses were euthanized without surgery.

At the time of hospital admission, most colic-affected horses had low AT activity and prolonged aPTT as laboratory criteria of DIC. In contrast, none of the colic-affected horses were thrombocytopenic, indicating that the platelet count was an insensitive indicator of DIC in this patient population (Table 1). When test results for colic-affected horses at the time of admission were examined for association among assays, the ranks of aPTT significantly correlated with the ranks of fibrinogen concentration ( $R_{sp} 0.49, P = .029$ ) and AT activity ( $R_{sp} -0.54, P = .015$ ).

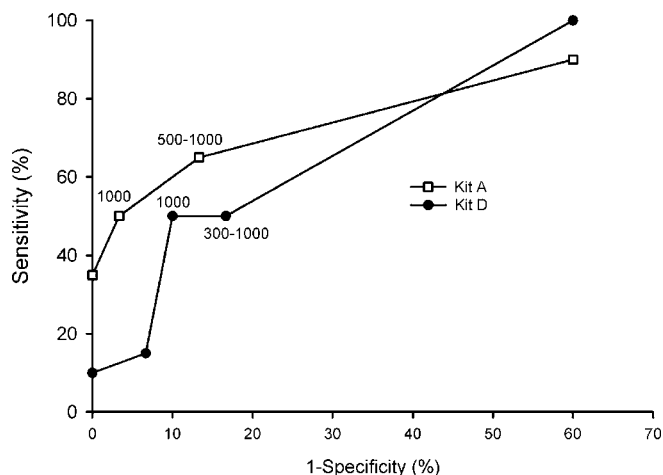
Serum and plasma FDP assays had low sensitivity (<50%) for diagnosis of DIC in colic-affected horses (Table 2). The serum FDP assay was also nonspecific, with positive values observed for almost half of all control horses. Furthermore, serum from 6 of the 13 FDP test-positive horses (43%) reacted at both dilutions (ie, concentration >40  $\mu g/mL$ ). Using 1000 ng/mL as a cut-off concentration, D-dimer kits A and D had the highest sensitivity and were similarly specific. On the basis of ROC curves, a cut-off concentration of 500 ng/mL for kit A improved sensitivity to 65% (95% CI, 42–88%), but decreased specificity to 87% (95% CI, 73–100%; however, the difference in these parameters was not statistically significant (Figure 2). The use of 300–1000 ng/mL and 1000 ng/mL cut-off concentrations for kit D produced the same sensitivity; however, the 1000 ng/mL cut-off concentration was slightly more specific. Therefore, the latter was considered optimal for this kit.

When kit results were compared with coagulation assay results in colic-affected horses at the time of admission, there

**Table 2.** Point estimates and 95% confidence intervals (CI) for sensitivity and specificity of fibrin(ogen) degradation product (FDP) and D-dimer assays for diagnosis of disseminated intravascular coagulation in horses with colic. Sensitivity was defined as the number of colic-affected horses (n=20) with positive FDP or D-dimer results at admission. Specificity was defined as the number of healthy horses (n=30) with negative FDP or D-dimer results.

Assay	Sensitivity (%)	95% CI	Specificity (%)	95% CI
Serum FDP*	37	13–61	57	37–76
Plasma FDP	15	0–33	100	98–100
D-dimer kit A	50	26–74	97	89–100
D-dimer kit B	20	0–40	100	98–100
D-dimer kit C	40	16–64	57	37–76
D-dimer kit D	50	26–74	90	78–100

\*Serum FDP assays were performed on samples from 29 healthy horses.



**Figure 2.** Receiver-operator characteristic curves for D-dimer results from use of latex-agglutination kit A and card immunofiltration kit D for samples from clinically healthy horses (n=30) and horses with severe colic (n=20). Numbers refer to cut-off D-dimer concentrations (ng/mL).

was a significant negative rank correlation between AT activity and D-dimer kit D ( $R_{sp} -0.67, P = .002$ ). When FDP and D-dimer results were compared in these horses, there was a significant positive rank correlation between D-dimer kits A and D ( $R_{sp} 0.62, P = .004$ ).

To determine whether any assayed variable at the time of admission was predictive of outcome in horses with colic, median values (excluding PT) were compared among the 3 study groups: healthy, colic survivor, and colic nonsurvivor. For PT, the proportions of horses with PT values above the reference interval were compared among groups. The colic nonsurvivors had significantly higher median aPTT and lower AT activity than did healthy or surviving colic-affected horses (Table 1). Because some healthy horses had AT activity below the reference limit of 86%, the proportions of surviving and nonsurviving colic-affected horses having AT values below those of healthy horses (74% lower limit) were compared. Significantly more nonsurvivors (8/9) than survivors (2/11) had AT activity <74% ( $P = .006$ , Fisher's exact test). This suggests that AT activity <74% is a negative prognostic indicator in horses with severe colic. There was no significant difference in the proportions of survivors (4/11) versus nonsurvivors (5/9) having prolonged PT; however, a significantly greater proportion of colic-affected horses (9/20) had prolonged PT, compared with that of healthy horses (0/30,  $P = .002$ ). D-Dimer concentrations measured by use of kits A and D were significantly higher in nonsurvivors, compared with healthy horses, but were not significantly different from concentrations in surviving colic-affected horses (Table 3).

When positive and negative results for individual horses in all groups were compared among FDP and D-dimer assays, discordant results were seen in 12–54% (6–27/50) of the horses, depending on the pairs of tests evaluated (Table 4). The highest concordance (88%, 44/50) was observed between D-dimer kits A and D ( $kappa = 0.67, P < .0001$ ), using the 1000 ng/mL cut-off concentration. These kits were similarly concordant (80%, 16/20,  $kappa = 0.60, P = .004$ ) when only

**Table 3.** Median serum fibrin(ogen) degradation product (FDP), plasma FDP, and D-dimer values at time of admission for healthy and colic-affected horses (separated by survival).\*

Assay	Healthy (n=30)†	Survivors (n=11)	Nonsurvivors (n=9)
Serum FDP (µg/mL)	<10 <sup>a</sup>	<10 <sup>a</sup>	10–40 <sup>a</sup>
Plasma FDP (µg/mL)	<5 <sup>a</sup>	<5 <sup>a</sup>	<5 <sup>a</sup>
D-dimer kit A (ng/mL)	250–500 <sup>a</sup>	250–500 <sup>ab</sup>	1000–2000 <sup>b</sup>
D-dimer kit B (ng/mL)	<500 <sup>a</sup>	<500 <sup>a</sup>	<500 <sup>a</sup>
D-dimer kit C (ng/mL)	<1000 <sup>a</sup>	<1000 <sup>a</sup>	<1000 <sup>a</sup>
D-dimer kit D (ng/mL)	300 <sup>a</sup>	300 <sup>ab</sup>	1000 <sup>b</sup>

\*Medians with common superscripts are not significantly different ( $P \geq .05$ ).

†Serum FDP assays were performed on samples from 29 healthy horses.

colic-affected horses were evaluated. The concordance of kits A and D in all groups decreased to 80% (40/50), when the 500 ng/mL cut-off concentration for kit A was used ( $kappa, 0.53, P < .0001$ ).

To determine whether assay results changed with time after admission, the median results at times 0 and 24 were compared for surviving horses (n = 11). For PT, paired data for a prolonged PT were compared for systematic bias in the direction of change. Surviving horses had significantly longer aPTT, lower AT activity, and lower platelet count at time 24, compared with time 0. Also, the results for D-dimer kits A and D were significantly higher at time 24, compared with time 0 (Table 5). There was no significant systematic change in the PT over time.

**Table 4.** Concordance (numbers in boldface type) between positive and negative results of serum fibrin(ogen) degradation product (FDP) and plasma FDP and D-dimer assays for all horses (n=50).\*

Assay	Plasma FDP		D-dimer Kit A		D-dimer Kit B		D-dimer Kit C		D-dimer Kit D		
	+	-	+	-	+	-	+	-	+	-	
Serum FDP†	+	<b>1</b>	19	<b>4</b>	16	<b>2</b>	18	<b>7</b>	13	<b>7</b>	13
	-	2	<b>27</b>	7	<b>22</b>	1	<b>28</b>	14	<b>15</b>	6	<b>23</b>
Plasma FDP	+		<b>2</b>	1	<b>0</b>	3	<b>1</b>	2	<b>2</b>	1	
	-		9	<b>38</b>	3	<b>44</b>	20	<b>27</b>	11	<b>36</b>	
D-dimer kit A	+			<b>3</b>	8	<b>6</b>	5	<b>9</b>	2		
	-			0	<b>39</b>	15	<b>24</b>	4	<b>35</b>		
D-dimer kit B	+				<b>3</b>	0	<b>2</b>	1			
	-				18	<b>29</b>	11	<b>36</b>			
D-dimer kit C	+					<b>7</b>	14				
	-					6	<b>23</b>				

\*+ indicates positive result (ie, serum FDP concentration  $\geq 10$  µg/mL, plasma FDP concentration  $\geq 5$  µg/mL, D-dimer concentration  $\geq 1000$  ng/mL). – indicates negative result (ie, serum FDP concentration <10 µg/mL, plasma FDP concentration <5 µg/mL, D-dimer concentration <1000 ng/mL).

†Serum FDP assays were performed on samples from 29 healthy horses (total comparisons done on results for 49 horses).

**Table 5.** Hemostasis test (median and range) and fibrin(ogen) degradation product (FDP) and D-dimer (median) results in surviving horses with colic (n=11) at the time of admission (time 0) and 24 hours later (time 24).\*

Parameter	Time 0	Time 24
aPTT (s)	55 <sup>a</sup> (41–78)	72 <sup>b</sup> (38–115)
Fibrinogen (mg/dL)	236 <sup>a</sup> (148–384)	292 <sup>a</sup> (163–605)
AT activity (%)	85 <sup>a</sup> (66–122)	61 <sup>b</sup> (54–100)
Platelet count ( $\times 10^9/L$ )	176 <sup>a</sup> (105–418)	150 <sup>b</sup> (107–242)
Serum FDP ( $\mu\text{g/mL}$ )	<10 <sup>a</sup>	<10 <sup>a</sup>
Plasma FDP ( $\mu\text{g/mL}$ )	<5 <sup>a</sup>	<5 <sup>a</sup>
D-dimer kit A (ng/mL)	250–500 <sup>a</sup>	>2000 <sup>b</sup>
D-dimer kit B (ng/mL)	<500 <sup>a</sup>	500–1000 <sup>a</sup>
D-dimer kit C (ng/mL)	<1000 <sup>a</sup>	<1000 <sup>a</sup>
D-dimer kit D (ng/mL)	300 <sup>a</sup>	1000 <sup>b</sup>

\*aPTT indicates activated partial thromboplastin time; AT, antithrombin activity.

Medians with common superscripts are not significantly different (comparing time 0 with time 24).

## Discussion

D-Dimer analysis has aided in the clinical management of equine colic<sup>12,14</sup>; however, the IF kit (kit D) used in the reported European studies is not commercially available in the United States. In our study, latex-agglutination kit A had the highest kappa value (concordance) with kit D and similarly favorable sensitivity and specificity values. On the basis of these findings, we conclude that kit A is the preferred latex-agglutination assay for detecting D-dimer in horses. Kit A could be used at either cut-off concentration (500 or 1000 ng/mL), although the higher cut-off concentration might provide better specificity because mean D-dimer values in healthy horses range from 551–875 ng/mL (as measured by ELISA).<sup>2,11,13</sup> The remaining kits had poor sensitivity and/or specificity and lacked even moderate concordance beyond chance for kit A or D. We acknowledge that test specificity was calculated from a group of healthy horses, rather than ill horses confirmed not to have DIC. The specificity values in this study, therefore, are likely to overestimate test specificities derived from clinical populations. The poor specificity point estimates of serum and plasma FDP assays and D-dimer kits B and C under these study conditions, however, obviate the need to further evaluate these tests as diagnostic indicators of DIC in horses with colic.

It is notable that the D-dimer concentration in healthy horses, as measured by use of ELISA by others<sup>2,11,13</sup> or latex-agglutination and IF assays by us, often is within the limit of analytic sensitivity for the commercial kits (kit A: 250 ng/mL, kit B: 500 ng/mL, kit C: 1000 ng/mL, kit D: 300 ng/mL). This contrasts with values for healthy people and dogs, with D-dimer concentration generally observed below the analytic sensitivity of the latex-agglutination assays.<sup>22,23</sup> The reason for this is unclear, but could be related to differences in baseline fibrinolytic activity of equine plasma or species differences in antibody reactivity. This finding affects how the D-dimer test

is performed and interpreted, because a positive agglutination reaction for undiluted equine plasma is an expected, rather than pathologic result. To avoid the time and cost involved in serial sample dilutions (as performed in this study), clinical samples analyzed by use of kit A could be diluted 1:2 or 1:4 with buffered saline to obtain cut-off concentrations of 500 and 1000 ng/mL, respectively. Positive agglutination at these dilutions would indicate D-dimer concentration above these cut-off concentrations.

Our results also indicate that serum and plasma FDP concentrations are not valid markers of pathologic fibrinolysis in horses. The serum FDP assay traditionally has been used for this purpose. However, this assay had low sensitivity and specificity, and results were positive for nearly 50% of the healthy horses (many of which had concentrations >40  $\mu\text{g/mL}$ ). Similar to that of previous studies,<sup>1–3,7</sup> we used 10  $\mu\text{g/mL}$  as a cut-off concentration for serum FDP concentration, although others used a slightly higher value of 20  $\mu\text{g/mL}$ <sup>24</sup> (suggesting that healthy horses can have serum FDP concentration >10  $\mu\text{g/mL}$ ). The poor specificity of the serum FDP kit might arise from residual fibrinogen in the test serum. The polyclonal antibodies in this kit cross-react with intact fibrinogen, necessitating its removal (accomplished by the thrombin-like activity of the *B atrox* venom in the collection tube).<sup>25</sup> It is possible that equine fibrinogen is resistant to the enzymatic activity of this venom, resulting in false-positive reactions. In contrast to its use in dogs with DIC,<sup>22,26</sup> the plasma FDP assay was uninformative in this study. However, we only evaluated 1 plasma FDP kit. It is possible that other available kits containing different antibody specificities for plasma FDPs would be useful in horses.

There was a striking lack of concordance among results of serum and plasma FDP assays, FDP and D-dimer assays, and the various D-dimer kits. Similar lack of concordance has been reported for canine studies.<sup>17,22,23</sup> Each commercial kit uses unique antibodies raised against the various human FDPs. The variation in kit performance we observed may be attributed to differences in antibody affinity and reactivity with the corresponding equine fibrin degradation fragments.

Our results support the findings of others<sup>12,14</sup> that D-dimer analysis is a useful ancillary test for diagnosis of DIC in horses with severe colic. Increased D-dimer concentration ( $\geq 1000$  ng/mL with use of kits A and D) was the third most frequent hemostatic abnormality (found in 50% of the colic-affected horses of our study), preceded by low AT activity (70%) and prolonged aPTT (55%). In contrast, none of our colic-affected horses were thrombocytopenic and few were hypofibrinogenemic, indicating that these criteria for diagnosis of DIC in other species do not aid in the diagnosis of DIC in horses with colic. This apparent species difference is in agreement with previously reported studies of horses with colic.<sup>1,3,24</sup> In our study, prolonged aPTT and high D-dimer concentration obtained by use of kits A and D were associated with low AT activity. The correlation between these results is not surprising because the thrombotic tendency of DIC is caused by systemic activation of thrombin, with resultant depletion of coagulation factors, neutralization and clearance of AT, and release of D-dimer from fibrin thrombi. In our study, the aPTT was correlated positively with fibrinogen

(ie, prolonged aPTT was associated with increasing fibrinogen) concentration. Fibrinogen is an acute-phase reactant protein in horses.<sup>27</sup> A concomitant increase in fibrinogen concentration and prolongation of aPTT (due to factor depletion) likely reflect the intensity of the initiating stimulus of DIC (ie, inflammation caused by endotoxemia).

It must be noted that there are conflicting data in the literature concerning D-dimer concentration in horses with colic. When measured by use of IF or immunoturbidometric assays, D-dimer concentration was higher in horses with colic, compared with that in clinically normal horses<sup>7,14</sup>; however when measured by use of ELISA, the concentration was lower in similar cohorts of horses.<sup>2</sup> This discrepancy is unexplained and raises questions as to the specificity and cross-reactivity of the D-dimer antibodies in the various assays. Further studies are needed to determine the specificity of the antibodies in the various kits or which degradation products exist in horses with DIC.

At the time of admission, median aPTT and AT activity were significantly different between survivors and nonsurvivors of our study, indicating that prolonged aPTT and low AT activity (particularly <74%) were negative prognostic indicators in horses with colic. This was similar to findings in previous reports.<sup>1,2,24,28</sup> In contrast, median D-dimer concentration was not significantly different between survivors and nonsurvivors, suggesting that high D-dimer concentration at the time of admission does not necessarily indicate a poor outcome in horses with colic. In contrast to our findings, D-dimer concentration (measured by use of the IF kit, kit D) was significantly higher in nonsurviving horses of 2 previous studies of horses with colic.<sup>14,15</sup> The addition of D-dimer testing to a logistic model containing heart rate and chloride concentration improved the predictive value of the model for distinguishing between survivors and nonsurvivors in one of these studies,<sup>14</sup> but was not confirmed by results of the second, more recent study.<sup>15</sup> However, in neither study were other hemostatic parameters measured. It is possible that had we tested more horses with colic and then evaluated D-dimer in series or parallel with the other hemostatic tests, D-dimer might have been revealed to be a useful prognostic indicator. However, on the basis of results of all studies performed to date, it does not appear that D-dimer adds sufficient prognostic information to justify the cost of running the test for this purpose.

Many of the test abnormalities found at admission became more pronounced over 24 hours, including increases in D-dimer concentration (using kits A and D). A sequential increase in aPTT and fibrinogen concentration was seen in all 7 surviving horses that underwent surgery. Similar findings have been reported in horses after colic surgery and were attributed to surgically induced inflammation (fibrinogen concentration) and the underlying colic condition (aPTT).<sup>12</sup> It is unlikely that the surgical procedure caused the sequential increase in D-dimer concentration in our horses, because increases also were observed in 2 of 4 horses treated medically (compared with 6 of 7 horses treated surgically), and surgery alone did not increase D-dimer concentration above reference intervals in a previous study.<sup>12</sup> We chose to evaluate D-dimer and FDP assays on the basis of test results only at the time of

admission for several reasons: 1) all nonsurviving horses were euthanized within 24 hours of admission, 2) treatment of surviving horses might affect the results,<sup>8,12</sup> and 3) prognostic information at the time of admission was more likely to influence immediate therapeutic decisions. In all nonsurviving horses, the decision for euthanasia was based on severity of clinical signs of disease and not on financial or other considerations, which could have introduced a bias unrelated to disease state.

In conclusion, our results indicate that a commercial latex-agglutination D-dimer assay can be used to support the diagnosis of DIC in horses with colic; however, results of serum and plasma FDP assays should not be considered valid indicators of abnormal fibrinolysis in this species. Of the tests evaluated in this study, the best negative prognostic indicators at admission for horses with colic were prolonged aPTT (>63 seconds) and low AT activity (<74%).

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