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Cardiovascular, Colloid Osmotic Pressure, and Hemostatic Effects of 2 Formulations of Hydroxyethyl Starch in Healthy Horses

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Background: Lower molecular weight and molar substitution formulations of hydroxyethyl starch (HES) solutions might maximize cardiovascular function and colloid osmotic pressure (COP) and minimize adverse effects on coagulation.

Hypothesis/Objectives: To compare effects of 1 low and 1 high molecular weight and molar substitution HES solution on cardiovascular variables, COP, and hemostasis in normal horses.

Animals: Eight healthy adult horses.

Methods: Randomized, crossover designed study: 10 mL/kg bolus of 6% HES (600/0.75) (hetastarch) (HS), 6% HES (130/0.4) tetrastarch (TS), and 0.9% NaCl (NS). Variables recorded included central venous pressure (CVP), noninvasive arterial blood pressure, packed cell volume (PCV), COP, and automated platelet analysis (CT).

Results: Central venous pressure was increased for 8 hours after all treatment (baseline = 8.4 ± 3.8; 8 hours = 10.3 ± 3.5 cm H_2O; $P < .001$). HS and TS produced an increase in systolic arterial pressure (HS = 109.1 ± 11.9; TS = 109.5 ± 10.9 mmHg) and mean arterial pressure (HS = 80.4 ± 13.0; TS = 82.3 ± 10.1 mmHg) compared to NS (SAP = 103.2 ± 13.2 [$P = .023$]; MAP = 74.2 ± 11.4 mmHg [$P = .048$]). PCV decreased transiently with HS (baseline = 37.1 ± 4.4%; 1.5 hours = 31.6 ± 3.9%) and TS (baseline = 38.4 ± 3.9%; 1.5 hours = 32.2 ± 3.3%), but not NS ($P = .007$). COP was greater with HS (1 hour; 24.0 ± 2.1 mmHg) and TS (8 hours; 25.9 ± 2.1 mmHg) than NS (1 hour = 20.8 ± 2.6; 8 hours = 22.9 ± 3.1 mmHg; $P < .001$). CT was greater at 8 (HS = 178.6 ± 36.9; TS = 121.9 ± 33.3; NS = 108.3 ± 23.6 seconds) and 24 hours (HS = 174.2 ± 41.7; TS = 100.8 ± 26.0; NS = 118.7 ± 38.7 seconds; $P < .001$) in horses receiving HS than TS or NS.

Conclusion and Clinical Importance: Both TS and HS resulted in more effective volume expansion and arterial pressure support than NS. TS produced a more sustained effect on COP with shorter duration of adverse effects on platelet function than HS.

Key words: Colloid therapy; Fluid therapy; Pharmacodynamics; Platelet function.

Hydroxyethyl starches (HES) are synthetic polymers of branched polysaccharide (amylopectin) with hydroxyethyl substitution. Solutions containing HES can be used to provide oncotic pressure to restore and maintain intravascular volume. During resuscitation for hypovolemia, synthetic colloids can be used to provide sustained volume expansion, compared to crystalloid fluids, which rapidly redistribute out of the vasculature.[1] In a study evaluating rapid preoperative volume resuscitation in horses with colic, administration of a synthetic colloid improved intraoperative cardiovascular indices to a greater extent and for a longer period of time than hypertonic saline.[2] Synthetic colloids can also be used to replace natural colloids that have been lost. Administration of synthetic colloids to horses with hypoproteinemia results in a significant increase in oncotic pressure.[a,3]

The adverse effects of synthetic colloid solutions include allergenicity, kidney injury, tissue accumulation, and interference with coagulation.[4] The effects on hemostasis are related to both dilution of and interference with coagulation factors, fibrinolysis, and platelets.[5,6] HES molecules might adhere to platelet membranes, and they might impair fibrinogen binding by direct effects on the GPIIb/IIIa fibrinogen receptor, or by preventing association of the receptor and ligand.[7] Effects are dose dependent and limit the maximum recommended dose of these products.[5] Coagulation impairments associated with administration of HES solution have been evaluated in humans,[5,8,9] dogs,[10,11] and horses.[3,12–14]

Abbreviations:

HES	hydroxyethyl starch
COP	colloid osmotic pressure
NS	0.9% saline
HS	6% hydroxyethyl starch 600/0.75 in 0.9% saline (hetastarch)
TS	6% hydroxyethyl starch 130/0.4 in 0.9% saline (tetrastarch)
SAP	systolic arterial blood pressure
MAP	mean arterial blood pressure
DAP	diastolic arterial blood pressure
CVP	central venous pressure
PCV	packed cell volume
ACT	Sonoclot®activated clotting time
CR	Sonoclot®clot rate
PF	Sonoclot®platelet function
vWF	von Willebrand factor
FVIII:C	coagulation factor VIII
PT	prothrombin time
aPTT	activated partial thromboplastin time
CT	closure time as measured by the PFA-100

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Multiple formulations of HES exist and are differentiated by the molecular weight (high, medium, and low) and molar substitution ratio (number of hydroxyethyl groups per glucose molecule) of the starch molecule, as well as the electrolyte and buffering composition of the solution.[5,15,16] Products with lower molecular weights have more oncotically active molecules at similar concentrations compared to higher molecular weight products.[17] Thus, the beneficial effects on the cardiovascular system and colloid osmotic pressure are greater for low molecular weight formulations. Plasma clearance is higher with lower molecular weight and lower molar substitution formulations. While this property decreases the risk for tissue accumulation, it also shortens the duration of effect.[17] Formulations with increased molecular weight and higher molar substitution ratios are more likely to interfere with coagulation.[4,18–20]

A low molecular weight and molar substitution HES solution (6% HES, 130 kD/0.4) (tetrastarch) has recently been approved for veterinary use.[b] *In vitro* comparison of this product to higher molecular weight and molar substitution HES (6% HES 600 kD/0.75 and 670 kD/0.75) (hetastarches) suggests that there is less impairment of coagulation by the low molecular weight and low molar substitution formulation.[14] This study was designed to compare the effects of a low and high molecular weight and molar substitution formulation of HES solution on cardiovascular variables, colloid osmotic pressure (COP), and hemostasis in normal horses. We hypothesized that the low molecular weight and molar substitution solution would have a similar effect on cardiovascular variables, cause a greater increase in COP, and cause less impairment of hemostasis.

Materials and Methods

Instrumentation

Eight clinically healthy adult horses (7 Quarter Horses and 1 Warmblood; 3 stallions and 5 mares; weight 498–597 kg; age 6–17 years) were studied in a randomized crossover study. Order of fluid administration was randomized using a random number generator.[c] Horses were determined to be healthy based on physical examination and observation. Horses were kept in stalls and allowed free access to food and water throughout the study period. They were restrained with a halter and lead rope during instrumentation, fluid administration, sampling, and monitoring. Horses were instrumented with 1 catheter in each jugular vein, one a 14 g 5¼" over-the-needle catheter[d] for administration of fluids and blood sampling, and the other a 7 French, 60 cm single lumen catheter[e] placed using a modified Seldinger technique, used for measurement of central venous pressure (CVP). An evaluation of length and of a characteristic venous pressure waveform was used to confirm the placement of the tip of this catheter. Catheters were flushed with a 50% dextrose solution if no sampling was to occur for longer than 2 hours in order to maintain patency without affecting coagulation testing, and with 0.9% saline for shorter sampling intervals. At the end of each fluid assessment, the catheters were removed and horses turned out in their normal pasture. There was a washout period of at least 1 week before the next fluid was administered.

Study Drug Administration

After instrumentation, horses were administered study fluid given IV using two 1 L size pressure infusers[f] inflated to a pressure of 300 mmHg. The study fluid was administered as rapidly as possible using this method, and the use of 2 infusers per horse allowed continuous infusion whereas the subsequent bag of fluids was spiked with the infusion set. Fluids were administered in the following dosages: 10 mL/kg 6% HES (600/0.75) in 0.9% saline[g] (hetastarch) (HS), 10 mL/kg 6% HES (130/0.4) in 0.9% saline[h] (tetrastarch) (TS), or 10 mL/kg 0.9% NaCl[i] (NS). During fluid administration, each horse was monitored for any changes in attitude or signs of colic. Rectal temperature, heart rate, respiratory rate, arterial blood pressure, and CVP were determined for each horse before starting the infusion (baseline), immediately after the infusion was complete (time 0), and 0.5, 1, 1.5, 8, and 24 hours after this time. Arterial blood pressure was measured using an oscillometric blood pressure monitor[j] and a 22–32 cm cuff placed around the tail base. To determine systolic, mean, and diastolic arterial blood pressure (SAP, MAP, and DAP, respectively), 3 readings were obtained at each time point, and the results averaged. CVP was measured in mm Hg, using a calibrated transducer[k] and multivariable monitor.[l] Three sequential readings were taken and then averaged for each time point. If a CVP tracing did not display the expected venous pressure waveform, a reading was not recorded.

Sampling

Blood was sampled from each horse: before fluid administration (baseline), at the end of the infusion (time 0), and 0.5, 1, 1.5, 8, and 24 hours after the end of the infusion. All samples were obtained from the 14 g jugular venous catheter. For each sampling period, 6 mL of blood was withdrawn from the catheter before study samples were obtained. Blood for platelet aggregometry was collected directly into syringes containing 3.2% sodium citrate,[m] for a final citrate:blood ratio of 1 : 9. Blood for coagulation factor, clotting times, viscoelastic coagulation, and automated platelet function analyzer analyses was collected using a 6 mL syringe[n] and immediately placed into three evacuated tubes[o] containing 3.2% citrate, again for a final citrate: blood ratio of 1 : 9. Blood samples for analysis of packed cell volume (PCV) were collected into an evacuated tube containing lyophilized EDTA[o] before analysis. This blood was also used to obtain platelet counts using an automated cell counter.[p] Whole blood was also collected into evacuated tubes containing lithium heparin[o] for evaluation of colloid osmotic pressure (COP), which was performed on heparinized plasma using a colloid osmometer.[q]

Platelet Function Analyses

Optical platelet aggregation[r] was performed at baseline, time 0, and 8 hours using final concentrations of ADP[s] and collagen[r] of 15 μM and 15 μg/mL, respectively, for activation as previously described.[21]

Citrated whole blood was also evaluated using an automated platelet function analyzer[t] that simulates a condition of high shear stress. A quantity of 800 μL of citrated whole blood is aspirated by vacuum across a narrow aperture. The aperture is coated with ADP and collagen, and generates a closure time (CT), or the time until the aperture is completely occluded by a platelet plug, causing an interruption of flow. CT was recorded in seconds and was assayed twice for each sample. The values from both runs were averaged for the purpose of statistical analysis.

Coagulation Analyses

Whole blood coagulation analysis was performed using a viscoelastic coagulation monitor,[u] using previously described techniques.[14,22,23] Briefly, samples were rested for 30 minutes at room temperature. Twenty microliters of 0.2M $CaCl_2$ was added to the warmed (37° C), glass bead-activated cuvette (gbACT+[v]) followed by 340 μL of citrated whole blood mixed by inversion five times. The viscoelastic analysis yielded 3 variables: activated clotting time (ACT) which represented the time until the initiation of clot formation; clot rate (CR), which represented the rate of clot formation; and platelet function (PF), a value calculated by the analysis software[v] using a proprietary algorithm.

Citrated whole blood that was not used for the above analyses was centrifuged at room temperature (22–24°C) and 1500 × *g* for 10 minutes. The plasma was collected and kept frozen at −80°C until batch analysis by the Cornell comparative coagulation laboratory. All samples were assayed for activities of von Willebrand factor (vWF) and coagulation factor VIII (FVIII:C). In addition, baseline and time 0 thawed frozen plasma samples were assayed for prothrombin (PT) and activated partial thromboplastin times (aPTT).

Statistical Analysis

Statistics were performed using commercially available statistical software.[w] A two-way ANOVA for repeated measures was used to assess the effect of treatment (HS, TS, NS), time, and the interactions between treatment and time. Data that were not normally distributed were log- or rank-transformed before analysis. When warranted (an interaction between time and treatment detected), multiple pairwise comparisons were done using the method of Holm–Sidak. Additionally, if an effect of treatment was detected, a pairwise comparison using the method of Holm–Sidak was performed to determine if there was a preexisting difference at baseline. For all analyses, $P < .05$ was considered statistically significant.

Results

With the exception of vWF and FVIII:C, none of the variables were significantly different between treatments at baseline. At baseline, horses receiving TS and HS had higher vWF activity than horses receiving NS and horses receiving HS had higher FVIII:C than horses receiving TS and NS.

Physical Examination (Table S1)

All horses tolerated all 3 infusions well, showing no signs of discomfort. There was a transient decrease in rectal temperature for all treatments. However, there was no significant difference between treatments (Fig 1A). Heart rate decreased for all treatments starting at the 8 hour time point. Overall, horses receiving HS had higher heart rates than those receiving NS (Fig 1B). There were no significant changes in respiratory rate over time or between treatments (Fig 1C).

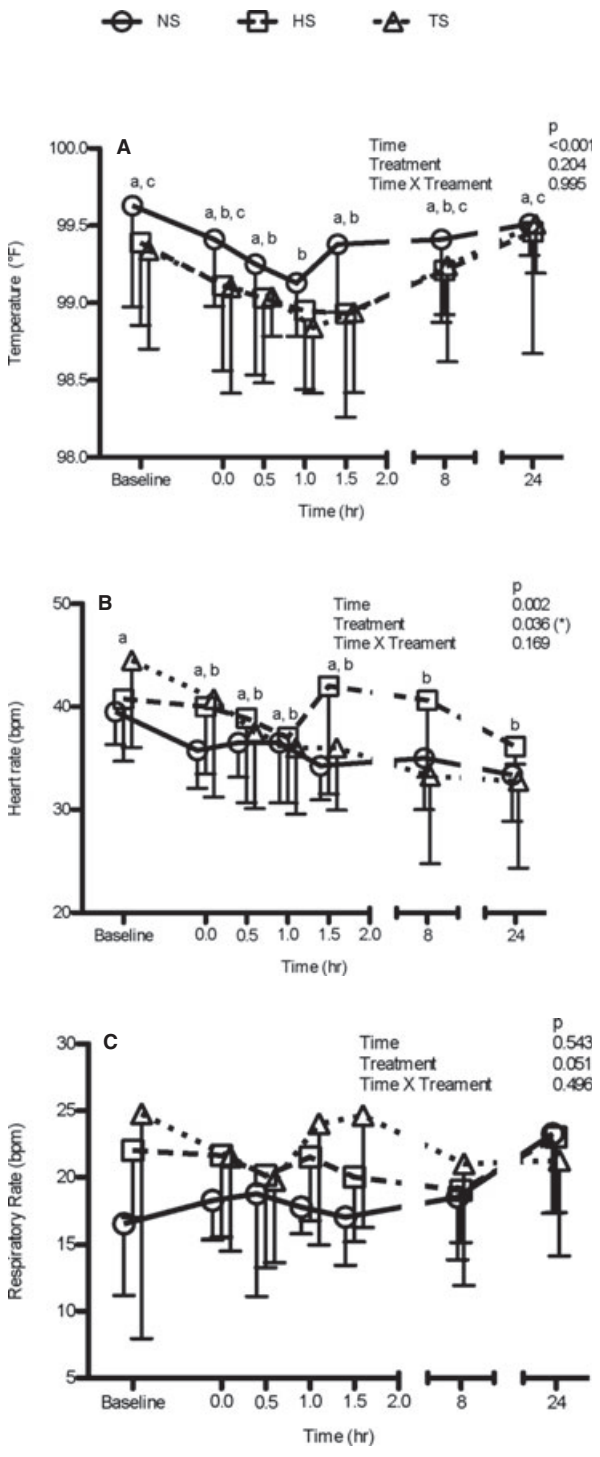

Fig 1. Temperature **(A)**, heart rate **(B)**, and respiratory rate **(C)** for each treatment group over time (mean and SD). NS = 0.9% saline; HS = 6% hydroxyethyl starch 600/0.75 in 0.9% saline (hetastarch); TS = 6% hydroxyethyl starch 130/0.4 in 0.9% saline (tetrastarch). Different lowercase letters indicate significant differences over time. * indicates that NS is significantly different from HS.

Cardiovascular Assessment (Table S2)

CVP was increased for 8 hours for all treatments. However, there was no significant difference between treatments (Fig 2A). MAP was increased at 0.5 hours compared to 24 hours. Overall, horses receiving TS had increased SAP, MAP, and DAP compared to

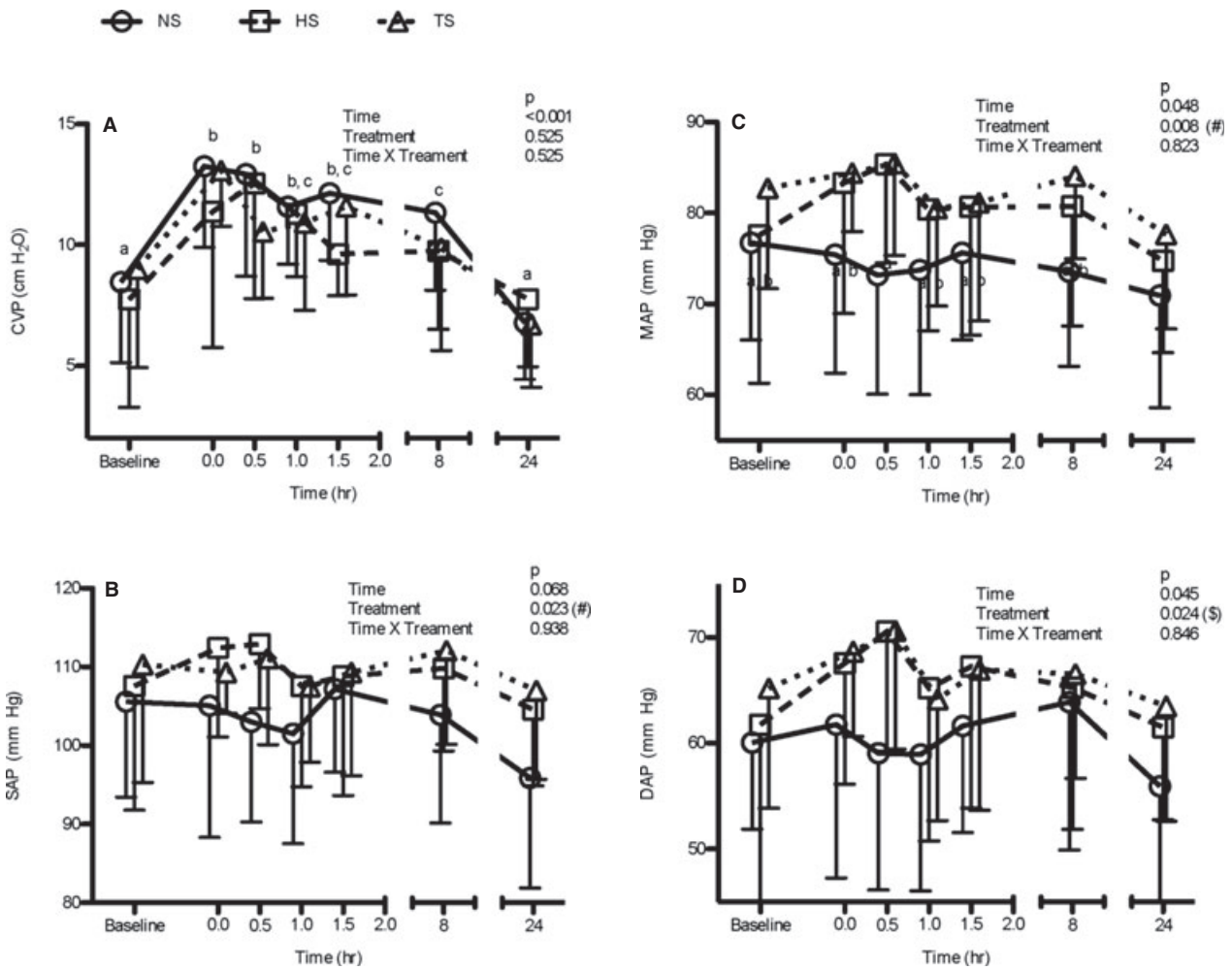

Fig 2. Central venous pressure (CVP) **(A)**, systolic arterial pressure (SAP) **(B)**, mean arterial pressure (MAP) **(C)**, and diastolic arterial pressure (DAP) **(D)** for each treatment group over time (mean and SD). NS = 0.9% saline; HS = 6% hydroxyethyl starch 600/0.75 in 0.9% saline (hetastarch); TS = 6% hydroxyethyl starch 130/0.4 in 0.9% saline (tetrastarch). Different lowercase letters indicate significant differences over time. # indicates that NS is significantly different from HS and TS. $ indicates that NS is significantly different from TS.

horses receiving NS and horses receiving HS had increased SAP and MAP compared to horses receiving NS (Fig 2B [SAP]; Fig 2C [MAP]; Fig 2D [DAP]).

Hemodilution and COP (Table S3)

Horses receiving TS and HS, but not NS, had a transient decrease in PCV from baseline after treatment. At 8 hours, horses receiving TS had an increased PCV compared to horses receiving HS and NS (Fig 3A). Horses receiving TS and HS had an increase in COP compared to baseline for 24 hours after treatment whereas horses receiving NS had a decrease in COP for 1 hour after treatment. Horses receiving TS and HS had increased COP compared to horses receiving NS for 8 hours and 1 hour, respectively (Fig 3B).

Coagulation and Platelet Function (Table S4)

With the exception of 1 horse in the HS group at 1.5 hours (75,000/μL), platelet counts were within instrument reference range (94–208,000/μL) throughout the study. There was a transient decrease in platelet count from baseline for all treatment groups. Overall, horses receiving HS had lower platelet counts than horses receiving NS (Fig 4).

PT and aPTT were increased compared to baseline for all treatment groups (Fig 5A [PT]; Fig 5B [aPTT]). Overall, horses receiving HS had increased aPTT compared to horses receiving TS. One horse had aPTT outside of the reference interval in all samples except for baseline when treated with TS. Three other horses had aPTT outside of the reference range. One horse had prolongation only for baseline TS, one had prolongation at baseline TS and at 1 hr HS, and one had prolongation at 1 hr TS and NS.

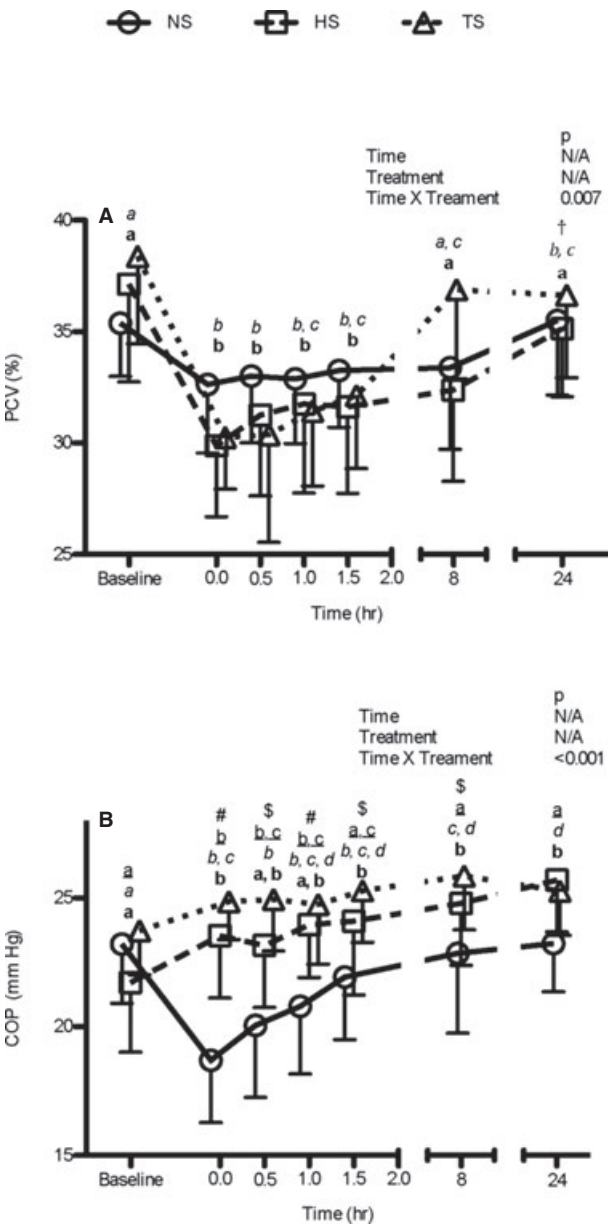

Fig 3. Packed cell volume (PCV) **(A)** and colloid osmotic pressure (COP) **(B)** for each treatment group over time (mean and SD). NS = 0.9% saline; HS = 6% hydroxyethyl starch 600/0.75 in 0.9% saline (hetastarch); TS = 6% hydroxyethyl starch 130/0.4 in 0.9% saline (tetrastarch). Different lowercase letters indicate significant differences over time. Letters are underlined font for NS, *italics font for HS*, and **bold for TS**. † indicates TS is significantly different from NS and HS. # indicates that NS is significantly different from HS and TS. $ indicates that NS is significantly different from TS.

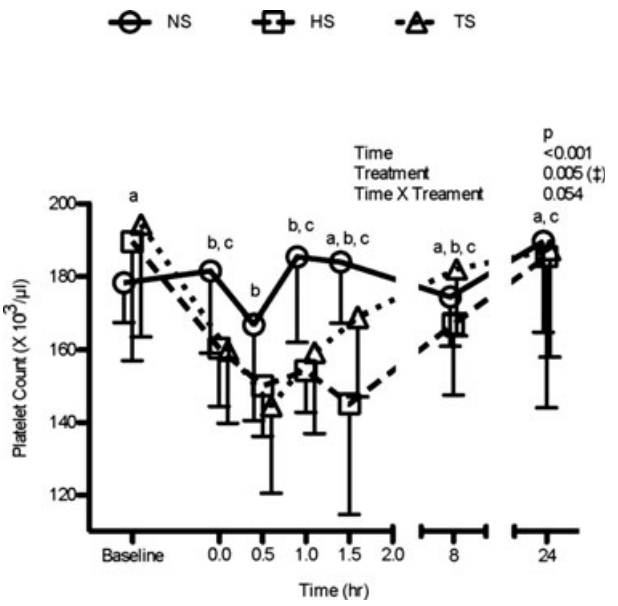

Fig 4. Platelet count for each treatment group over time (mean and SD). NS = 0.9% saline; HS = 6% hydroxyethyl starch 600/0.75 in 0.9% saline (hetastarch); TS = 6% hydroxyethyl starch 130/0.4 in 0.9% saline (tetrastarch). Different lowercase letters indicate significant differences over time. ‡ indicates that NS is significantly different from HS.

In horses receiving TS and HS, vWF was decreased compared to baseline for 8 and 24 hours, respectively. In horses receiving NS, vWF was increased compared to first 1.5 hours after treatment at 24 hours (Fig 6A). In horses receiving TS, there was a transient decrease after treatment in FVIII:C from baseline and compared to 8 and 24 hours. In horses receiving HS, there was a persistent decrease after treatment in FVIII:C for 24 hours. In horses receiving NS, FVIII:C was decreased for 1 hour after treatment compared to 24 hours. At 8 and 24 hours after treatment, horses receiving TS had decreased FVIII:C compared to horses receiving TS and NS. At 24 hours after treatment, horses receiving NS had decreased FVIII:C compared to horses receiving TS (Fig 6B).

There was a transient decrease in ACT compared to baseline for all treatment groups. However, there were no significant differences in ACT between treatments (Fig 7A). CR was increased 0.5 hours compared to 24 hours for all treatment groups. However, there were no significant differences in CR between treatments (Fig 7B). PF was decreased at 1 hour compared to 24 hours in horses receiving TS and at 1.5 hours compared to baseline in horses receiving HS. At 24 hours, horses receiving TS had increased PF compared to horses receiving TS (Fig 7C).

CT was increased from baseline for 8 hours in horses receiving TS and for 24 hours in horses receiving HS. In horses receiving HS, the increase in CT was most pronounced at 8 and 24 hours and was greater than the CT in horses receiving TS and NS at these time points (Fig 8).

With the exception of 1 horse in the NS group at 8 hours (77,000/µL), platelet counts in platelet rich plasma for optical aggregometery were 100,000–300,000/µL (range of concentrations shown to not affect the results of aggregometry[24]). The slope of the platelet aggregation curve with collagen activation was decreased in horses receiving HS compared to horses

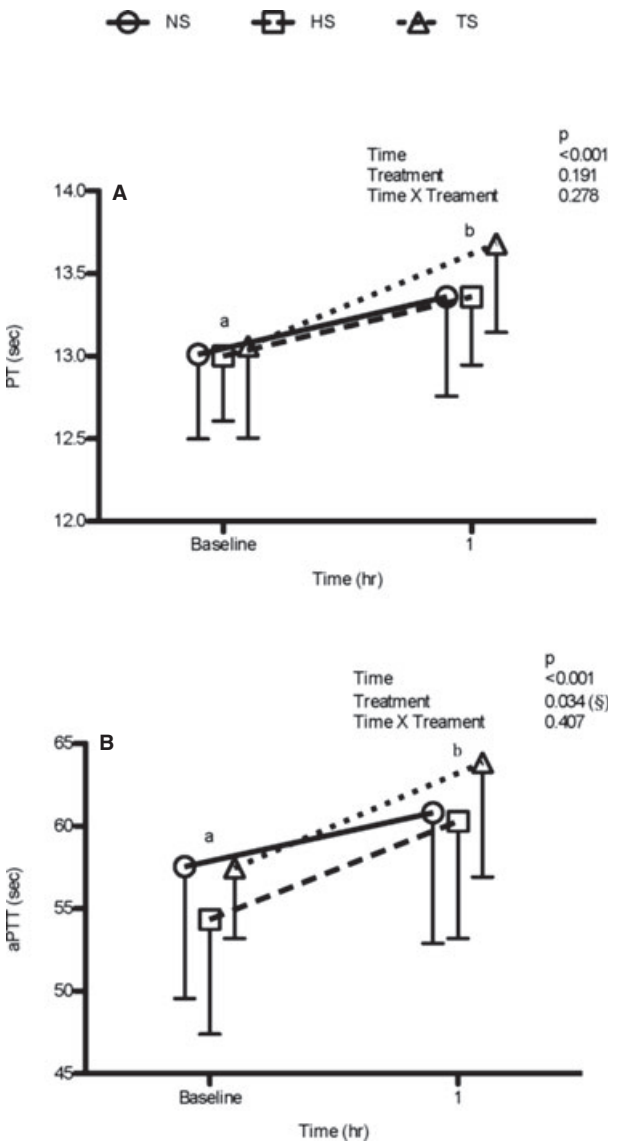

Fig 5. Prothrombin time (PT) **(A)** and activated partial thromboplastin time (aPTT) **(B)** for each treatment group over time (mean and SD). NS = 0.9% saline; HS = 6% hydroxyethyl starch 600/0.75 in 0.9% saline (hetastarch); TS = 6% hydroxyethyl starch 130/0.4 in 0.9% saline (tetrastarch). Different lowercase letters indicate significant differences over time. § indicates that HS is significantly different from TS.

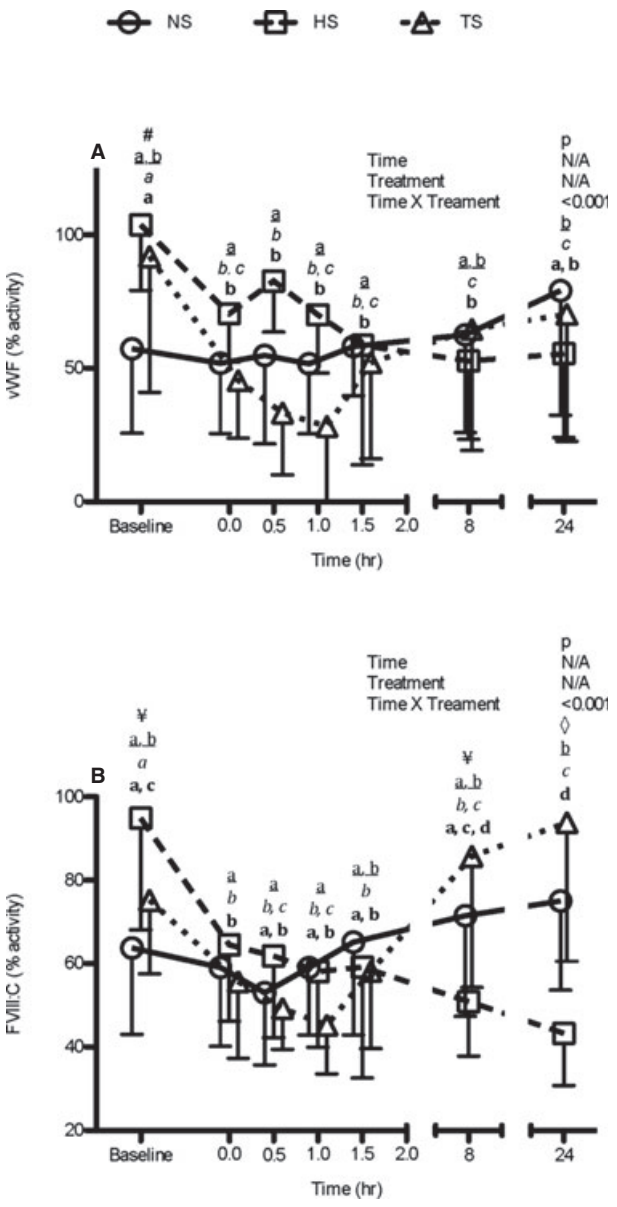

Fig 6. von Willebrand factor (vWF) **(A)** and factor VIII activity (FVIII:C) **(B)** for each treatment group over time (mean and SD). NS = 0.9% saline; HS = 6% hydroxyethyl starch 600/0.75 in 0.9% saline (hetastarch); TS = 6% hydroxyethyl starch 130/0.4 in 0.9% saline (tetrastarch). Different lowercase letters indicate significant differences over time. Letters are underlined font for NS, *italics font for HS*, and **bold for TS**. # indicates that NS is significantly different from HS and TS. ¥ indicates that HS is significantly different from NS and TS. ◊ indicates NS is significantly different from HS, which is significantly different from TS.

receiving NS. However, there was no significant difference over time (Fig 9A). There were no significant changes in percent aggregation with collagen activation over time or between treatments (Fig 9B). There were no significant changes in slope or percent aggregation with ADP activation over time or between treatments (slope Fig 9C; percent aggregation Fig 9D).

Discussion

The IV bolus administration of HES solutions to horses at a dose of 10 mL/kg was well tolerated and resulted in vascular volume expansion (as shown by an increase in CVP and blood pressure and a decrease in PCV) and maintenance or increase of plasma COP. Administration of all 3 fluid types resulted in a transient increase in CVP, MAP, PT, and aPTT and decrease in temperature, ACT, and platelet count. Changes in SAP and MAP were more pronounced with TS and HS than with NS administration and changes in DAP were more pronounced with TS than

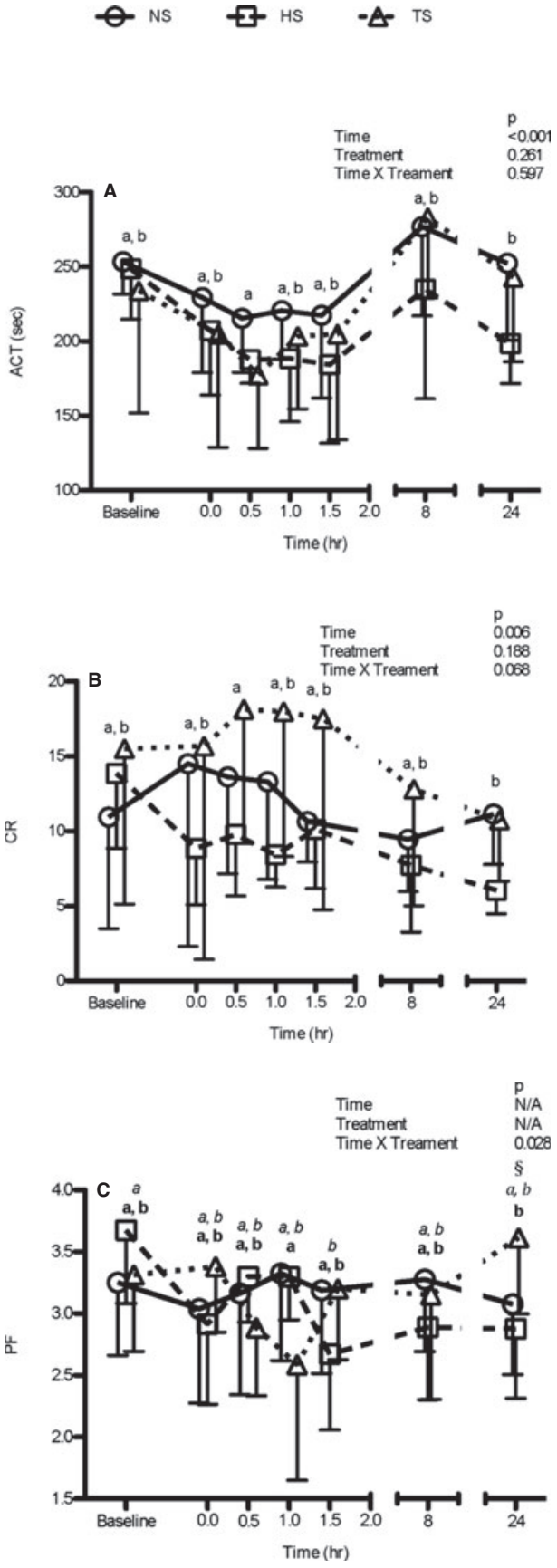

Fig 7. Sonoclot® activated clotting time (ACT) **(A)**, Sonoclot® clot rate (CR) **(B)**, and Sonoclot® platelet function (PF) **(C)** for each treatment group over time (mean and SD). NS = 0.9% saline; HS = 6% hydroxyethyl starch 600/0.75 in 0.9% saline (hetastarch); TS = 6% hydroxyethyl starch 130/0.4 in 0.9% saline (tetrastarch). Different lowercase letters indicate significant differences over time. When a significant interaction between treatment and time was found, letters are underlined font for NS, *italics font for HS*, and **bold for TS**. § indicates that HS is significantly different from TS.

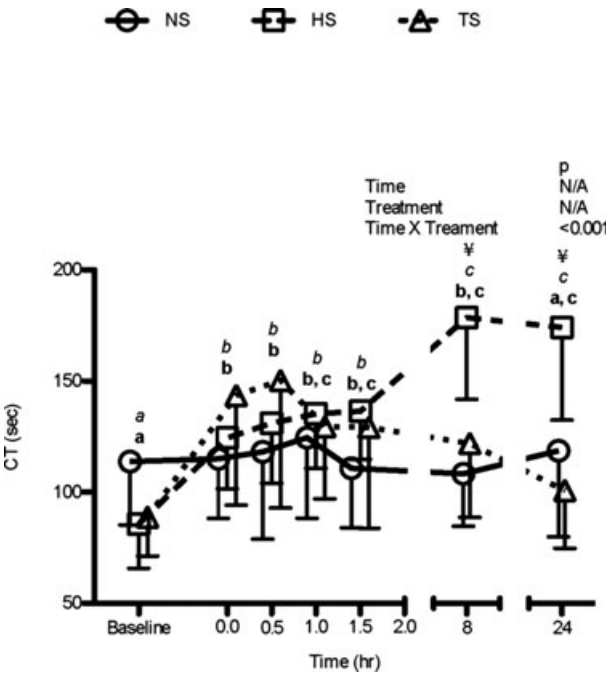

Fig 8. Closure time (CT) for each treatment group over time (mean and SD). NS = 0.9% saline; HS = 6% hydroxyethyl starch 600/0.75 in 0.9% saline (hetastarch); TS = 6% hydroxyethyl starch 130/0.4 in 0.9% saline (tetrastarch). Different lowercase letters indicate significant differences over time. Letters are underlined font for NS, *italics font for HS*, and **bold for TS**. ¥ indicates that HS is significantly different from NS and TS.

with NS administration. Changes in aPTT were greater in the TS group than in the HS group although neither HES group was different from the NS group. Overall, effects on plasmatic coagulation testing (PT, aPTT) were minimal. However, some horses in each treatment group at baseline and at 1 hour did have aPTT values slightly prolonged beyond the reference range. Changes in platelet function measured by the automated platelet function analyzer were more pronounced, with significant prolongations in CT seen in both HES groups, and persisting to 24 hours after treatment with HS. Measures of vWF and fVIII:C activity also decreased in the HES groups and mirrored the changes in CT. Whereas no changes were seen in the total amplitude of platelet aggregation under the low-shear conditions of optical aggregometry, a difference in the rate of platelet aggregation

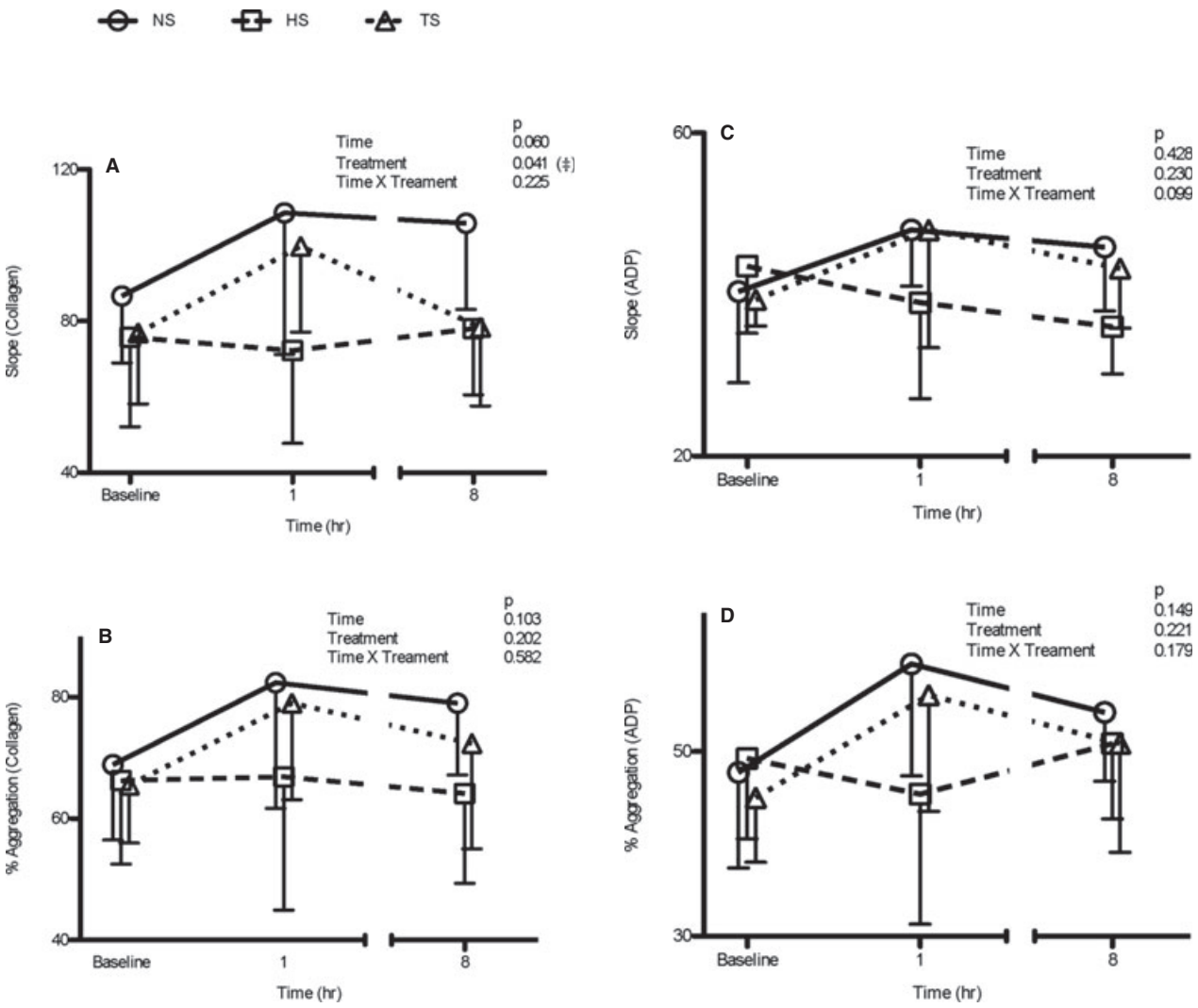

Fig 9. Slope **(A)** and percent aggregation **(B)** of platelet aggregation curve after collagen activation and slope **(C)** and percent aggregation **(D)** of platelet aggregation curve after ADP activation for each treatment group over time (mean and SD). ‡ indicates that NS is significantly different from HS.

(reported as the slope of the aggregation) was detected between the NS group and the HS group, with the HS group aggregating more slowly than the NS group with collagen activation.

Although there were some changes observed in physiologic variables, most were maintained within reference ranges for healthy adult horses during the administration of all study fluids and are not considered clinically relevant. The significant increases in CVP from baseline values are indicative of the volume effects of the initial fluid bolus, although the persistence of the increased CVP in all groups through 8 hours after infusion was unexpected. Rapid redistribution of NS from the vascular compartment to the interstitial fluid space was expected to result in a more rapid normalization of CVP compared to the HES containing fluids, but this was not seen. This might be a result of the fact that this study was performed in euvolemic horses with normal vascular tone and capillary reflection coefficient. The relatively lower values for all arterial blood pressures in the NS group could reflect the persistence in the vasculature of the HES solutions, and CVP might be an insensitive method to monitor for subtle shifts in overall vascular volume.

Both HES solutions resulted in an increase in plasma COP compared to NS. This was most apparent at 0 hour, and was maintained in the TS group through the 8 hour time point. After the 0 hour time point, the HS group was only significantly higher than the NS group at the 1 hour time point, which reflects a rapid redistribution of the NS bolus and normalization of the COP in combination with a possibly weaker colloid effect of HS (COP = 25–30 mmHg) compared to TS (COP = 36 mmHg),[5] although the 2 HES groups were not statistically different from each other for the remainder of the study period. The COP for all groups had normalized by 24 hours after the bolus.

A significant decrease in PCV or hemodilution was seen in both HES solution groups, but not in the NS group suggesting improved volume expansion with HES solutions. The hemodilution was shorter lived in the TS group compared to the HS group, which still had a significantly lower PCV (compared to baseline) at 8 hours. This finding suggests a greater volume expansion with HS and contradicts the longer duration of increased COP and more pronounced effects on blood pressure observed in the TS group. It also contradicts expected changes in vascular volume following the 2 HES solutions. Increased COP causes translocation of fluid volume from the interstitial space into the vascular space. It is expected that HS would result in a vascular volume expansion of about 100% of the infused volume, whereas TS would result in an expansion to 130% of the infused volume.[5] The reason for this discrepancy is unknown. However, it is notable that despite statistical significance the changes in PCV observed were of small magnitude and there was significant overlap in recorded PCV between the groups at all time points.

Hemodilution might also have contributed to the decreases in circulating platelet count and prolongation of PT and aPTT seen after the bolus of all 3 fluids. However, other factors might also have played a role considering the platelet count in the NS group was only significantly higher compared to the HS group and not the TS group and PT and aPTT in the HS and TS groups were not significantly different from the NS group. Changes in PT and aPTT have been variable in previous studies of horses receiving HES solutions,[3,12,13] but were considered to be attributable to hemodilution in one of the studies.[3] The aPTT at the 1 hr time point was prolonged beyond the reference interval in 3 horses (1 horse for all 3 treatments, 1 horse for TS and NS treatment, and 1 horse for HS treatment). However, 2 of these also had baseline values that were above the reference interval for 1 or more treatments. When comparing the overall value of aPTT after treatment, the mean of the horses treated with TS was outside of the reference interval and longer than when treated with HS. The reason for this is unclear, but could be related to greater volume expansion or direct effects on coagulation. Additionally, the magnitude of the difference was small and there was considerable overlap in results of aPTT in all groups making the clinical significance of the statistical differences questionable.

The decrease in vWF and fVIII activities as a result of HES administration is a phenomenon that has been reported in many human[25] and some veterinary[3,14] studies, and likely contributes to the impaired ability of the platelets to aggregate, especially under conditions of high shear. The most frequently cited hypothesis for the drop in these factors is that the HES molecules bind to the vWF/fVIII complex and speed excretion from circulation. In some human studies, lower MW HES cause a lesser decrease in these factors than larger MW HES.[25,26] In this study, an effect of HES solution formulation appeared minimal with the only differences between HS and TS occurring for FVIII:C at 8 and 24 hours.

Although a variety of instruments are available for viscoelastic coagulation testing, only 1 instrument was chosen for this study. The instrument used for this study was chosen over thromboelastography because more significant changes were found during previous *in vitro* testing in horses.[14] Coagulation effects were not as prominent using viscoelastic testing as previous *in vitro* work in horses[14] and humans.[18,27] In this study, the only variable that changed associated with treatment was PF. The mean ACT at 8 hours was significantly prolonged compared to all prior time points, but there was not a significant difference between groups, or changes seen between baseline and earlier time points. Because platelet function, plasmatic coagulation factors, and fibrinogen all contribute to the formation of clots described by whole blood viscoelastic coagulation monitoring, a prolonged ACT and decreased CR were expected, but not seen. Coagulation in this test occurred via the intrinsic pathway, activated using glass beads, and it is possible that the strong activator was able to overwhelm any decreased coagulation function (similar to that seen with the aPTT assay). In addition, the viscoelastic coagulation monitor is also a low-shear assay, and if the effects of HES molecules are most pronounced in the context of platelet attachment during high shear conditions, large effects on viscoelastic coagulation would not be expected.

The significant prolongations in CT peaked in the TS group at 0.5 hours, whereas the peak prolongation was seen at 8 hours in the HS group. The significant CT prolongation from baseline in the HS group was maintained through 24 hours, whereas the CT in the TS group had returned to baseline values by 24 hours. This time course might be a manifestation of the more rapid excretion of the smaller TS molecule, or of the persistence of the larger HS molecules. The 6% HS solution is a polydisperse solution, containing a wide range of molecular sizes, with an average MW of 600 kD but a range of 450–800 kD. According to the package insert, at least 80% of the HES polymers in a bag of 6% HS are within a MW range of 20–2,600 kD.[x] The smaller MW molecules are excreted rapidly (the renal threshold for excretion is 60 kD or less) whereas the medium size molecules are broken down by α-amylase and excreted over a 12–24 hour period after a bolus, the larger molecular weight molecules can remain in circulation or bound in body tissues for days after the initial bolus.[17] The lower MW TS has an average MW of 130 kD and a range from 110 to 150 kD,[y] and has a clearance that is 26- to 31-fold faster than HS,[17] with a terminal half-life after a single bolus of about 12 hours. The persistence of the prolonged CT for up to 24 hours might also have been seen because the larger HES molecules are more likely to impair platelet function.[20] This property might have also resulted in the relatively slower rate of aggregation of platelets seen in the HS group with optical aggregometry.

While the prolongations of clotting times were relatively mild, it was statistically significant, and raises the concern that prolonged administration of HES products in clinically ill or coagulopathic horses might result in prolonged coagulation times and possible clinical bleeding. The same might be true for horses with preexisting thrombocytopenia or thrombocytopathia given the changes observed in vWF, FVIII:C, PF, and CT. Therefore, the benefits of artificial colloids should be weighed with the risk of bleeding in these animals. Monitoring of coagulation times (PT and aPTT), platelet function, and for signs of clinical bleeding might be indicated in horses where prolonged usage is anticipated, especially if there is a high index of suspicion for coagulopathy.

Several limitations of the study should be considered when interpreting the findings. First, this study was performed in healthy horses and might not reflect the complications associated with the use of these fluids in horses with naturally occurring hypoproteinemia or hypovolemia. Second, in clinical cases, HES might also be administered as a constant rate infusion, rather than by bolus administration. It is possible that accumulation of HES might occur when administered as a constant rate infusion resulting in more pronounced adverse effects. Based on the pharmacokinetics from human studies, it might be expected that constant rate infusion of the TS product would result in less long-term accumulation than HS.[17] Third, this study focused on the potential adverse effects of HES solutions on coagulation and primarily on platelet function with minimal assessment of secondary coagulation. We elected to focus on platelet function because previous studies had found minimal changes in traditional coagulation testing with HES administration.[3,12,13] However, additional coagulation tests, such as fibrinogen and d-dimers or fibrin (ogen) degradation products, could have provided additional insight into the effects of HES solutions on coagulation.

Overall, our results support the use of HES solutions for volume expansion and colloid support in horses, but clinicians should be aware and consider the potential for adverse effects on secondary coagulation and platelet function. When comparing the 2 formulations evaluated, hemodynamic effects were similar with the 2 HES solutions, but more pronounced than with NS. However, TS might be preferable to HS because it produced a more sustained effect on COP with shorter duration of adverse effects on platelet function than HS.

Footnotes

[a] Hackett E, Kuhnmuench T. Colloidal support of horses with naturally occurring gastrointestinal diseases; effect of product formulation. J Vet Emerg Crit Care 2010;20(S1):A18 (abstract)
[b] VetStarch™, Abbott Laboratories, Abbott Park, IL
[c] Urbaniak GC, Plous S. (2011). Research Randomizer (Version 3.0) [Computer software]. http://www.randomizer.org/
[d] Milacath, Mila International, Erlanger, KY
[e] Arrow Teleflex, Reading, PA
[f] Mason Tayler, Buffalo, NY
[g] Hespan, B. Braun Medical Inc, Bethlehem, PA
[h] Voluven, Fresenius Kabi, Bad Homburg, Germany
[i] Hospira Inc, North Chicago, IL
[j] Dinamap, Critikon Inc, Tampa, FL
[k] Transpac IV, Hospira
[l] SurgiVet® Advisor, Smiths Medical, Norwell, MA
[m] Sigma-Aldrich, St. Louis, MO
[n] Kendall Monoject, Tyco Healthcare, Mansfield, MA
[o] Vacutainer, Becton Dickinson, Franklin Lakes, NJ
[p] CBC/Diff, Heska Corp., Loveland, CO
[q] Wescor 4420, Wescor Inc, Logan, UT
[r] Model 700, Chronolog Corp, Havertown, PA
[s] Chronolog Corp
[t] PFA-100, Siemens Healthcare, Tarrytown, NY
[u] Sonoclot, Sienco Inc, Arvada, CO
[v] Signature viewer, Sienco
[w] SigmaPlot, Systat Software, Inc, San Jose, CA
[x] Package insert Hespan, B. Braun Medical Inc, Bethlehem, PA
[y] Package insert Voluven, Fresenius Kabi, Bad Homburg, Germany

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References

1. Silverstein DC, Aldrich J, Haskins S, et al. Assessment of changes inblood volume in response to resuscitative fluid administration. J Vet Emerg Crit Care (San Antonio) 2005;15:185–192.

2. Hallowell GD, Corley KT. Preoperative administration of hydroxyethyl starch or hypertonic saline to horses with colic. J Vet Intern Med 2006;20:980–986.

3. Jones PA, Tomasic M, Gentry PA. Oncotic, hemodilutional, and hemostatic effects of isotonic saline and hydroxyethyl starch solutions in clinically normal ponies. Am J Vet Res 1997;58:541–548.

4. Jungheinrich C. The starch family: Are they all equal? Pharmacokinetics and pharmacodynamics of hydroxyethyl starches. Transfus Altern Transfus Med 2007;9:152–163.

5. Van der Linden P, Ickx BE. The effects of colloid solutions on hemostasis. Can J Anaesth 2006;53(6 Suppl.):S30–S39.

6. de Jonge E, Levi M. Effects of different plasma substitutes on blood coagulation: A comparative review. Crit Care Med 2001;29:1261–1267.

7. Deusch E, Gamsjager T, Kress HG, Kozek-Langenecker SA. Binding of hydroxyethyl starch molecules to the platelet surface. Anesth Analg 2003;97:680–683.

8. Kozek-Langenecker SA. Effects of hydroxyethyl starch solutions on hemostasis. Anesthesiology 2005;103:654–660.

9. Martin G, Bennett-Guerrero E, Wakeling H, et al. A prospective, randomized comparison of thromboelastographic coagulation profile in patients receiving lactated Ringer's solution, 6% hetastarch in a balanced-saline vehicle, or 6% hetastarch in saline during major surgery. J Cardiothorac Vasc Anesth 2002;16:441–446.

10. Wierenga JR, Jandrey KE, Haskins SC, Tablin F. In vitro comparison of the effects of two forms of hydroxyethyl starch solutions on platelet function in dogs. Am J Vet Res 2007;68:605–609.

11. Smart L, Jandrey KE, Kass PH, et al. The effect of Hetastarch (670/0.75) in vivo on platelet closure time in the dog. J Vet Emerg Crit Care (San Antonio) 2009;19:444–449.

12. Pantaleon LG, Furr MO, McKenzie HC, Donaldson L. Effects of small- and large-volume resuscitation on coagulation and electrolytes during experimental endotoxemia in anesthetized horses. J Vet Intern Med 2007;21:1374–1379.

13. Schusser GF, Rieckhoff K, Ungemach FR, et al. Effect of hydroxyethyl starch solution in normal horses and horses with colic or acute colitis. J Vet Med A Physiol Pathol Clin Med 2007;54:592–598.

14. Blong A, Epstein K, Brainard B. In vitro effect of three formulaitons of hydroxyethyl starch solutions on coagulation and platelet function in horses. Am J Vet Res 2013;74:712–720.

15. Treib J, Baron JF, Grauer MT, Strauss RG. An international view of hydroxyethyl starches. Intensive Care Med 1999;25:258–268.

16. Salmon JB, Mythen MG. Pharmacology and physiology of colloids. Blood Rev 1993;7:114–120.

17. Jungheinrich C, Neff TA. Pharmacokinetics of hydroxyethyl starch. Clin Pharmacokinet 2005;44:681–699.

18. Konrad CJ, Markl TJ, Schuepfer GK, et al. In vitro effects of different medium molecular hydroxyethyl starch solutions and lactated Ringer's solution on coagulation using SONOCLOT. Anesth Analg 2000;90:274–279.

19. Westphal M, James MF, Kozek-Langenecker S, et al. Hydroxyethyl starches: Different products–different effects. Anesthesiology 2009;111:187–202.

20. Franz A, Braunlich P, Gamsjager T, et al. The effects of hydroxyethyl starches of varying molecular weights on platelet function. Anesth Analg 2001;92:1402–1407.

21. Brainard BM, Epstein KL, LoBato D, et al. Effects of clopidogrel and aspirin on platelet aggregation, thromboxane production, and serotonin secretion in horses. J Vet Intern Med 2011;25:116–122.

22. Epstein KL, Brainard BM, Giguère S, et al. Serial viscoelastic and traditional coagulation testing in horses with gastrointestinal disease. J Vet Emerg Crit Care 2013;23:504–516.

23. Babski DM, Brainard BM, Krimer PM, et al. Sonoclot evaluation of whole blood coagulation in healthy adult dogs. J Vet Emerg Crit Care 2012;22:646–652.

24. Kingston JK, Bayly WM, Sellon DC, et al. Effects of sodium citrate, low molecular weight heparin, and prostaglandin E1 on aggregation, fibrinogen binding, and enumeration of equine platelets. Am J Vet Res 2001;62:547–554.

25. Gandhi SD, Weiskopf RB, Jungheinrich C, et al. Volume replacement therapy during major orthopedic surgery using Voluven (hydroxyethyl starch 130/0.4) or hetastarch. Anesthesiology 2007;106:1120–1127.

26. Jungheinrich C, Sauermann W, Bepperling F, Vogt NH. Volume efficacy and reduced influence on measures of coagulation using hydroxyethyl starch 130/0.4 (6%) with an optimised in vivo molecular weight in orthopaedic surgery: A randomised, double-blind study. Drugs R D 2004;5:1–9.

27. Casutt M, Kristoffy A, Schuepfer G, et al. Effects on coagulation of balanced (130/0.42) and non-balanced (130/0.4) hydroxyethyl starch or gelatin compared with balanced Ringer's solution: An in vitro study using two different viscoelastic coagulation tests ROTEMTM and SONOCLOTTM. Br J Anaesth 2010;105:273–281.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Temperature, heart rate, and respiratory rate results for time and treatment.

Table S2. CVP, DAP, MAP, and SAP results for time and treatment.

Table S3. PCV and COP results for time and treatment.

Table S4. Coagulation and platelet function test results for time and treatment.