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In vitro effects of lactated Ringer's solution, hypertonic saline, hydroxyethyl starch, hypertonic saline/hydroxyethyl starch, and mannitol on thromboelastographic variables of canine whole blood

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Abstract

Objective: To assess the in vitro effects of crystalloid and colloid IV fluids on the thromboelastographic (TEG) variables of canine whole blood.

Design: In vitro experimental study.

Setting: Veterinary teaching hospital.

Animals: Twenty-two healthy dogs.

Intervention: Citrated whole blood samples collected from healthy dogs were diluted with 3.4% hypertonic saline (HTS 3.4), 7% hypertonic saline (HTS 7), and 20% mannitol at 8% and 16% dilutions; hydroxyethyl starch 130/0.4 (HES 130/0.4) at 16% dilution; lactated Ringer's solution (LRS) at 16%, 33%, and 66% dilutions; and HTS 7-HES 130/0.4 at 25% and 50% dilutions. Kaolin-activated TEG analysis was concurrently performed on diluted and control (undiluted) samples.

Measurements and Main Results: Dilution of canine whole blood with LRS compared to control reduced α angle and MA at both 33% ($P = 0.009$ and $P = 0.011$, respectively) and 66% dilution ($P < 0.001$ and $P < 0.001$, respectively), and prolonged K time at 66% dilution ($P = 0.003$). At 16% dilution, HTS 3.4, prolonged R time ($P = 0.007$), while mannitol, a fluid iso osmolar to HTS 3.4, prolonged K time ($P = 0.006$), reduced α angle ($P < 0.001$), MA ($P = 0.046$), and LY60 ($P = 0.015$). At 8% dilution, HTS 7, a fluid of high osmolarity and tonicity, prolonged R time ($P = 0.009$) and reduced MA ($P = 0.015$), while all measured TEG variables were altered at the 16% dilution ($P < 0.01$ for all variables). HES 130/0.4 reduced α angle ($P = 0.031$) and MA ($P = 0.001$) and increased LY60 ($P < 0.001$) at 16% dilution. Comparing different fluid types, HES 130/0.4 and HTS 3.4 had no to minor, mannitol intermediate, and HTS 7 profound effects on TEG variables ($P < 0.05$) when compared to LRS at the same dilution.

Conclusions: In vitro dilution of canine whole blood with commonly used IV fluids leads to thromboelastographic changes consistent with hypocoagulability in a dose dependent manner for all

Abbreviations: HES, hydroxyethyl starch; HES 130/0.4, hydroxyethyl starch with an average molecular weight of 130kDa and molecular substitution of 0.4; HTS 3.4, 3.4% hypertonic saline; HTS 7, 7% hypertonic saline; LRS, lactated Ringer's solution; LY 60, percent of clot lysis at 60 minutes; MA, maximum amplitude in mm; TEG, thromboelastography.

fluid types tested. Viscoelastic changes are also influenced by fluid characteristics, specifically tonicity and osmolarity.

KEYWORDS
coagulation, dogs, dilutional coagulopathy, fluid therapy, viscoelastic testing

1 | INTRODUCTION

Resuscitative fluid therapy is used to rapidly replenish lost intravascular volume, improving tissue perfusion and potentially reverse shock. Fluid therapy therefore plays a fundamental role in treating dogs in hemorrhagic shock.[1] However, a consequence of fluid therapy is interference with hemostasis, which has a detrimental effect in trauma patients that are already hemostatically compromised by overconsumption and loss of coagulation factors from hemorrhage.[1,2] In addition, acidemia and hypothermia, both a consequence of hemorrhagic shock, can reduce platelet and clotting factor activity.[1-6] Fluid resuscitation may therefore contribute to further hemorrhage and continued hemodynamic de-stabilization, leading to increased blood transfusion requirements and mortality.[7,8] Current guidelines for resuscitating human patients in hemorrhagic shock subsequent to trauma include hypotensive and hemostatic resuscitation.[9,10] This involves restriction of isotonic crystalloid use and early transfusion of blood products including red blood cells, platelets, and fresh frozen plasma.[8-10] The limited availability of blood products for resuscitation of dogs in hemorrhagic shock and continued reliance on both crystalloids (isotonic and hypertonic) and synthetic colloids for intravascular fluid expansion means that an understanding of the effects of these fluids on coagulation in dogs is ideal.

Intravenous fluids may disrupt hemostasis by dilution of clotting factors and platelets.[11,12] This most commonly occurs with isotonic crystalloid fluids such as lactated Ringer's solution (LRS) and 0.9% sodium chloride because large volumes of these fluids are often administered to achieve resuscitative endpoints.[12] Although hemostatic dysfunction in trauma patients may be influenced by factors other than fluid administration, the incidence of coagulopathies in human trauma patients was found to increase in proportion to the volume of isotonic crystalloid fluids received prior to hospital admission. Coagulopathies were observed in >40%, >50%, and >70% of patients after 2, 3, and 4 L of fluids were administered, respectively.[13,14] The harmful effects of large volumes of IV isotonic crystalloids were demonstrated in a recent meta-analysis where restricted fluid resuscitation reduced morbidity and mortality in people suffering from hemorrhagic shock secondary to trauma when compared to liberal fluid resuscitation.[7]

The characteristics of specific fluid types might also directly affect hemostasis. For example, hydroxyethyl starches (HES) can affect hemostasis by a number of mechanisms including impaired platelet adhesion and aggregation, decreased circulating von Willebrand factor concentration and Factor VIII:C activity, and hyperfibrinolysis.[15-18] Experimental studies in dogs have also shown impaired platelet function[18-20] and hypocoagulable changes in viscoelastic variables following HES administration.[19,21–23] Similarly, 2 recent in vitro studies in dogs found that hyperosmotic fluids like hypertonic saline or mannitol affected hemostasis by interfering with platelet function and elicit changes in viscoelastic variables.[22,24] The tonicity or osmolality of fluids may be an important modulator of a fluid's effect on coagulation. However, the hemostatic effects of different hypertonic fluids at various dilutions and in direct comparison to previously examined fluids, such as HES and isotonic crystalloids, have not been investigated together in one study to the authors' knowledge.

The objective of this study was to determine the effect of dilution of canine whole blood with clinically relevant doses of common IV fluids on TEG variables. Our hypothesis was that in vitro dilution of canine whole blood from healthy dogs with IV fluids will induce dose-dependent changes in TEG variables consistent with hypocoagulability. Further, we hypothesized that the characteristics of the fluids, such as its tonicity and osmolality, will have effects on TEG variables in addition to those of dilution alone.

2 | MATERIALS AND METHODS

2.1 | Animals

Animal use for this study was approved by the Faculty of Veterinary and Agricultural Sciences' animal ethics committee. Informed consent was obtained from all pet owners. Dogs were included into the study if they weighed >6 kg and had no detectable systemic disease based on history, physical examination, and normal hematology, plasma biochemistry analysis, prothrombin time, and activated partial thromboplastin time results. Dogs were excluded from the study if they had received any corticosteroids, nonsteroidal anti-inflammatory drugs, antithrombotics, antimicrobials, or blood products in the 30 days prior to enrollment.

2.2 | Blood sample collection

A total of 4.5 mL of whole blood was collected using a 1-inch, 21-gauge needle into a 5 mL syringe by single venipuncture of the jugular vein. Blood was distributed directly into the collecting tubes in the following order; three 3.2% sodium-citrate tubes (1 mL)* for TEG and fibrinogen analysis, 1 EDTA tube (0.5 mL)† for hematology‡, and 1 lithium-heparin tube (1 mL)§ for plasma biochemical analysis¶. Up to 4 blood samples were collected from an individual dog. For the second, third, and fourth samples taken from the same dog, only 2 mL of whole blood was collected into two 3.2% sodium-citrate tubes for TEG analysis.

2.3 | Experimental groups and sample allocation

The fluid dilution percentages for this study were designed to mimic the effect of clinically relevant fluid doses administered to dogs with an initial blood volume of 90 mL/kg and an assumed loss of half of this volume. Furthermore, fluid types were selected to examine the influence of fluid characteristic (eg, tonicity, osmolality, or colloidality) at a constant dilution. A total of 12 experimental groups were studied, each characterized by a specific fluid dilution regimen. An 8% dilution, equivalent to a 4 mL/kg fluid bolus, was evaluated for 3.4% hypertonic saline (HTS 3.4)[#], 7% hypertonic saline (HTS 7)[‖], and 20% mannitol[**]. A 16% dilution, equivalent to an 8 mL/kg fluid bolus, was evaluated for LRS[††], HTS 3.4, HTS 7, 20% mannitol, and hydroxyethyl starch 130/0.4 (HES 130/0.4)[‡‡]. A 33% dilution, equivalent to a 16 mL/kg fluid bolus, and a 66% dilution, equivalent to a 32 mL/kg fluid bolus, were evaluated with LRS only. A solution that combined HTS 7 and HES 130/0.4 at a volume to volume ratio of 1:2 was tested at a 25% dilution, equivalent to 4 mL/kg bolus of HTS 7 and 8 mL/kg bolus with HES 130/0.4, and a 50% dilution, equivalent to 8 mL/kg fluid bolus with HTS 7 and 16 mL/kg fluid bolus with HES 130/0.4. For each experimental group, 6 samples were processed. Before blood collection, each blood sample was allocated to a specific experimental group by a random draw. Each experimental group was marked on 6 small pieces of folded paper. These were placed in a box for a blind draw without replacement. A new draw was performed with each dog entering the study. The investigator collecting the blood samples and conducting the TEG analysis was not blinded to the study group.

2.4 | TEG analysis

Blood sample collection and processing for TEG analysis followed the Partnership on Rotational ViscoElastic Test Standardization recommendations for veterinary viscoelastic testing.[25] Analysis with TEG was performed using a 2-channel 5000 series TEG analyzer[§§] to acquire the following parameters: R time, K time, α angle, MA, and LY 60. Control (undiluted) and the allocated treatment group (protocolized dilution) samples were run concurrently on the 2 channels of the TEG machine. All blood samples were held at room temperature for 30 minutes prior to processing. For diluted blood samples, a predetermined volume of fluid was pipetted into the kaolin tube[¶¶] followed immediately by addition of citrated blood to a total volume of 1 mL. For example, an 8% dilution was achieved by adding 80 μL of fluid to 920 μL of whole blood. After inverting the kaolin tube 5 times, 340 μL of the diluted blood was pipetted to a prewarmed TEG cup[##] containing 20 μL of 0.2 M calcium chloride and TEG processing immediately started. For the controls, 1 mL of the citrated blood was pipetted into a kaolin tube and the sample was then processed in an identical manner to the diluted blood samples. All TEG assays were run until the analyzer returned LY 60 results. Data were collected through TEG analytical software, downloaded, and entered into an Excel spreadsheet[‖‖] for further statistical manipulation. All TEG assays were performed by the same investigator.

2.5 | Platelet counts and fibrinogen concentrations

Baseline platelet counts and fibrinogen concentrations were determined in all dogs included in the study. This was performed only once per dog even if several samples were collected from the same animal. EDTA blood samples were processed using an automated analyzer[‡] for a platelet count. The platelet count was checked manually on a blood smear if the automated platelet count was below 150×10^9/L (150×10^3/μL). For determination of fibrinogen concentrations, citrated blood samples were centrifuged[***] at 4°C at $1{,}500 \times g$ for 5 minutes within 1 hour of blood collection. The separated plasma was collected in a plain polystyrene tube and stored at −20°C for up to 15 months. All plasma samples were processed in a single batch by an automated processor[†††] using the modified Clauss method[‡‡‡].

2.6 | Statistical analysis

An a priori power analysis was calculated to determine the sample size required to identify a change of at least 20% in the TEG variable MA between experimental and control samples with the alpha and beta error level set at 5 % and 20%, respectively.

Descriptive statistical analyses were used to present the results, with continuous data either expressed as mean ± SD or median and interquartile range, as appropriate. Values for MA were plotted as individual raw data points with group wise mean ± SD for overall visualization of central tendency and spread of the measurements. Differences between experimental and respective control) group were determined by a paired *t*-test or a Wilcoxon signed-rank test, as appropriate. For testing for differences between experimental groups (eg, between different dilutions and different fluid types), data for each TEG variable were first assessed for normality using fitted value plots. Skewed data were found for R time, K time and LY 60 variables. These TEG variables were log transformed prior to further analysis. A residual maximum likelihood variance component analysis to establish predicted means for TEG variables, independent of animal or sample order, was established by utilizing a linear mixed model, with experimental group (eg, fluid type and dilution) treated as fixed effect and dog identification and sample number from each dog as random effects. Least significant differences between experimental group data for the respective TEG variable were then calculated at the 5% level and comparison between groups executed. The procedure was repeated for each TEG variable across all experimental groups. Platelet count, fibrinogen level, and PCV were assessed as covariates of MA and α angle by allocating them as fixed effects in the linear mixed model described above. Genstat[§§§] and JMP[¶¶¶] were used for statistical analysis. $P < 0.05$ was considered statistically significant.

3 | RESULTS

Twenty-two healthy dogs, 9 neutered females and 13 neutered males with ages ranging from 1 to 13 years, were included in the study. The study population was composed of 17 purebred dogs represented by

TABLE 1 Comparison of thromboelastographic measurements between diluted and control samples

			TEG Variable				
Fluid type	**Dilution**	***N***	**R-time (min)**	**K-time (min)**	**α angle (degree)**	**MA (mm)]**	**LY 60 (%)**
Control	**N/A**	72	1.6 (0.6)	0.88 (0.2)	80.5 (2.3)	57.6 (5.0)	10.2 (5.9)
LRS	**16%**	6	1.5 (0.3)	1.1 (0.4)	78.8 (4.1)*	58.8 (5.7)	7.7 (3.9)
	33%	6	1.9 (0.5)	1.4 (0.7)	76.5 (4.3)§	48.9 (6.0)*	18.3 (17.5)
	66%	6	2.9 (2.5)	5.0 (1.8)*	64.4 (2.3)‖	29.7 (3.5)‖	21.7 (17.5)
HTS 3.4	**8%**	6	1.8 (0.9)	0.9 (0.1)	80.5 (2.2)	55.9 (8.26)	7.8 (1.7)
	16%	6	2.4 (0.8)§	1.1 (0.3)	78.0 (3.7)	55.9 (8.26)	6.4 (3.7)
HTS 7	**8%**	6	2.6 (0.8)§	1.4 (0.8)	75.3 (8.3)	52.9 (5.8)*	4.0 (2.4)
	16%	6	9.6 (4.6)‖	5.6 (1.6)‖	42.5 (9.6)‖	31.0 (5.1)‖	24.9 (4.9)§
Mannitol	**8%**	6	1.8 (0.4)	1.3 (0.6)	77.9 (4.1)	53.7 (9.8)	10.6 (5.3)
	16%	6	1.5 (0.7)	1.8 (0.5)*	71.1 (3.1)‖	48.8 (7.5)*	1.8 (1.5)*
HES 130/0.4	**16%**	6	1.5 (0.5)	1.1 (0.5)	77.4 (5.4)*	54.7 (4.9)*	11.8 (1.8)§
HTS 7-HES 130/0.4	**25%**	6	3.7 (1.8)*	2.3 (2.0)	68.9 (14.2)	45.1 (7.0)§	7.8 (4.5)
	50%	6	10.6 (3.8)§	9.5 (3.1)§	30.5 (11.3)‖	20.9 (3.0)‖	20.0 (7.9)‖

LRS, lactated Ringer's solution; HTS 3.4, 3.4% hypertonic saline; HTS 7, 7% hypertonic saline; HES 130/0.4, hydroxyethyl starch 130/0.4. Data are presented as mean (SD).

*$P < 0.05$ between experimental and control group;

§$P < 0.01$ between experimental and control group;

‖$P < 0.001$ between experimental and control group; control values represent the combined data of all control samples analyzed ($n = 72$).

12 different breeds and 5 dogs of mixed breeds. Platelet count was 242 ± 71.5 × 10^9/L (242 ± 72 × 10^3/μL), fibrinogen concentration was 4.65 ± 1.26 μmol/L (158 ± 43 mg/dL), and the PCV was 0.46 ± 0.5 L/L (46 ± 5%).

3.1 | Effect of dilution on TEG variables

When compared to controls (Table 1), dilution of canine whole blood with LRS decreased the α angle at all dilutions (16% [$P = 0.020$], 33% [$P = 0.009$], 66% [$P < 0.001$]), decreased the MA (33% [$P = 0.011$], 66% [$P < 0.001$]) (Figure 1) and prolonged the K time (66% [$P = 0.003$]). Dilution with HTS 3.4 was associated with a prolongation of R time (16% [$P = 0.007$]) only. HTS 7 prolonged R time (8% [$P = 0.009$], 16% [$P < 0.001$]) and K time (16% [$P < 0.001$]), decreased α angle (16% [$P < 0.001$]) and MA (8% [$P = 0.015$], 16% [$P < 0.001$]), and increased LY 60 (16% [$P = 0.004$]). Dilution of canine whole blood with 20% mannitol was associated with prolongation of K time (16% [$P = 0.006$]), decreased α angle (16% [$P < 0.001$]) and MA (16% [$P = 0.046$]), and decreased LY 60 (16% [$P = 0.015$]). HES 130/0.4 decreased the α angle (16% [$P = 0.031$]) and the MA (16% [$P = 0.034$]), and increased LY 60 (16% [$P < 0.001$]). Dilution of canine whole blood with HTS 7-HES 130/0.4 prolonged R time (25% [$P < 0.05$], 50% [$P < 0.01$]) and K time (50% [$P < 0.01$), decreased α angle (50% [$P < 0.001$]) and MA (25% [$P<0.01$], 50% [$P < 0.001$]), and increased LY 60 (50% [$P = 0.01$]).

All TEG variables were significantly altered at higher dilutions compared to lower dilutions with the fluid types LRS (16% compared to 66% dilution [$P < 0.05$]), HTS 7 (8% compared to 16% dilution [$P < 0.05$]), and HTS 7-HES 130.0.4 (25% compared to 50% dilution [$P < 0.05$]) causing prolongations in R and K time, decreased α angle and MA, and increased LY 60 (Table 2). Twenty percent mannitol also prolonged K time, decreased α angle, and decreased LY 60 when compared between 8% and 16% dilution ($P < 0.05$) (Table 2).

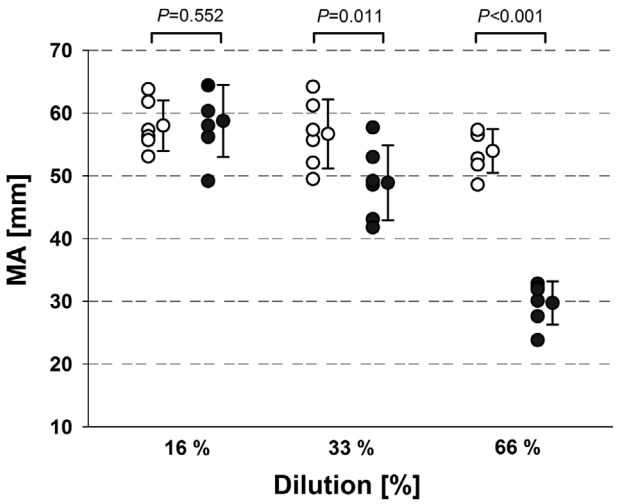

FIGURE 1 Effect of replacing 16%, 33%, and 66% of canine whole blood with LRS on maximum amplitude (MA). Control groups were not different among each other ($P = 0.30$). Dots represent individual measurements, bars and whiskers the mean and standard deviation. Open circles, control (undiluted) samples; Solid circles, experimental (diluted) samples. $P < 0.05$ was considered statistically significant

3.2 | Effect of fluid type on TEG variables

To assess the effect of fluid type on TEG variables, dilution with LRS at the 16% concentration was used as the reference and all other fluid

TABLE 2 Comparison of thromboelastographic measurements between dilution percent and between fluid types

			TEG Variable				
Fluid type	**Dilution**	**N**	**R-time (min)**	**K-time (min)**	**α angle (degree)**	**MA (mm)**	**LY60 (%)**
Control	**N/A**	72	1.5 (0.93)	0.8 (0.1)	80.4 (2.73)	56.85 (6.63)	9.15 (4.7)
LRS	**16%**	6	1.5 (0.45)	0.9 (0.35)	78.85 (4.05)	59.15 (6.73)	7.85 (2.5)
	33%	6	1.7 (0.5)*	1.2 (0.95)	73.45 (6.35)	45.85 (6.93)*	12.6 (2.25)
	66%	6	1.6 (1.05)*	4.45 (1.55)*	64 (2.55)*	31 (3.9)*	15.45 (6.23)*
HTS 3.4	**8%**	6	1.5 (1.2)	0.8 (0)	80.3 (2.25)	55.93 (8.3)	7 (1.78)
	16%	6	1.95 (0.95)§	2.1 (1.7)§	78.55 (5.3)	55.65 (6.55)	5.3 (5)
HTS 7	**8%**	6	2.55 (1.18)	1 (0.3)	77.95 (3.9)	48.83 (5.68)	0.55 (0.775)
	16%	6	8.7 (7.35)*,§	5.95 (2.6)*,§	43.7 (16.05)*,§	28.25 (6.1)*,§	24.4 (4.58)*,§
Mannitol	**8%**	6	1.85 (0.45)	1 (0.78)	79.05 (6.73)	53.9 (5.45)	9 (6.18)
	16%	6	1.15 (1)	1.85 (0.78)*,§	70.05 (5)*,§,‖	48.6 (13.25)§,‖	1.5 (1.98)*,§,‖
HES 130/0.4	**16%**	6	1.35 (0.48)	0.85 (1.76)	79.05 (1.6)	52.65 (7.15)	4.65 (1.38)
HTS 7-HES 130/0.4	**25%**	6	3.2 (1.88)	1.65 (0.63)	74.05 (7.83)	45.8 (5.55)	1.1 (3.1)
	50%	6	9.4 (2.45)*	11.3 (5.9)*	30.55 (8.23)*	21.05 (4.08)*	21.9 (12.05)*

LRS, lactated Ringer's solution; HTS 3.4, 3.4% hypertonic saline; HTS 7, 7% hypertonic saline; HES 130/0.4, hydroxyethyl starch 130/0.4.
Data presented as median (interquartile ranges).
*$P < 0.05$ compared with low and high dilution between each fluid types;
§$P < 0.05$ compared with LRS at equivalent (16%) dilution;
‖$P < 0.05$ comparing iso-osmolar HTS 3.4 and mannitol solution.

types were compared at the same dilution (Table 2). HTS 7 causes the most consistent changes affecting all TEG variables including prolongations in R time and K time, decreased α angle and MA, and increased LY 60 ($P < 0.05$). HTS 3.4, in contrast, caused a prolongation in R time and K time only ($P < 0.05$). Dilution with 20% mannitol, iso-osmolar to HTS 3.4, caused prolongations in K time, decreased α angle and MA, and decreased LY60 ($P < 0.05$). When comparing the iso-osmolar fluids, 20% mannitol decreased the α angle, decreased the MA, and decreased the LY 60 ($P < 0.05$) when compared to HTS 3.4 (Figure 2).

3.3 | Effect of the HTS 7-HES 130/0.4 combination

When comparing a 25% dilution of HTS 7-HES 130/0.4 to a 33% dilution of LRS, all TEG variables were significantly affected ($P < 0.05$). A 25% dilution with HTS 7-HES 130/0.4 caused prolongations in R time and K time, and decreased α angle and MA ($P < 0.05$) when compared with HES 130/0.4 alone (Figure 3). When comparing to HTS 7 at 8% dilution, K time was prolonged, α angle decreased, and MA was decreased ($P < 0.05$) (Table 3).

3.4 | Covariates

Fibrinogen concentration and the platelet count had a small but significant impact on the TEG variable MA. An increase in fibrinogen concentration by 1.26 μmol/L (100 mg/dL) was associated with an increase in MA of 7.6 mm ($P < 0.001$) and each additional 10×10^9/L (10×10^3/μL) platelets was associated with an increase in MA of 0.3 mm ($P = 0.048$).

4 | DISCUSSION

In vitro dilution of canine whole blood causes hypocoagulable changes in TEG variables compared to control with all fluid types examined. Dilution with increasing fluid volumes resulted in a greater magnitude of changes on coagulation compared to lower volume dilutions. Independent of this volume dependent dilution effect, high tonicity of the dilution fluid, such as with HTS 7, resulted in further compromise of coagulation. Different fluids of comparable osmolarity did not have identical effects on coagulation suggesting additional, non osmolarity dependent mechanisms.

Analysis with TEG provides information on alterations in viscoelastic properties of blood as clotting progresses over time.[26,27] It utilizes whole blood and so can provide information about the interaction of cell surfaces and platelet effects during clotting and more closely reflects the cell based model of coagulation.[26] The main variables obtained by TEG include R time, representing the initiation of blood clotting, K time and α angle, representing the dynamics of clot formation, MA, representing the overall clot strength achieved, and LY 30 or LY 60, representing the percentage of clot breakdown at 30 and 60 minutes.[26] The variable MA is a direct function of the maximum dynamic properties of fibrin and platelet bonding resulting from the contribution of platelets and to a lesser effect from fibrin to clot strength.[27,28] This is commensurate with our findings that MA is influenced by platelet count and fibrinogen levels. Hence, decreased platelet counts or fibrinogen levels caused by hemodilution will likely influence MA, leading to reduced clot strength.

In vitro fluid dilution of canine whole blood effects TEG variables in a dose dependent manner. This is seen with all fluid types tested, but the effect of dilution itself was most apparent and most relevant

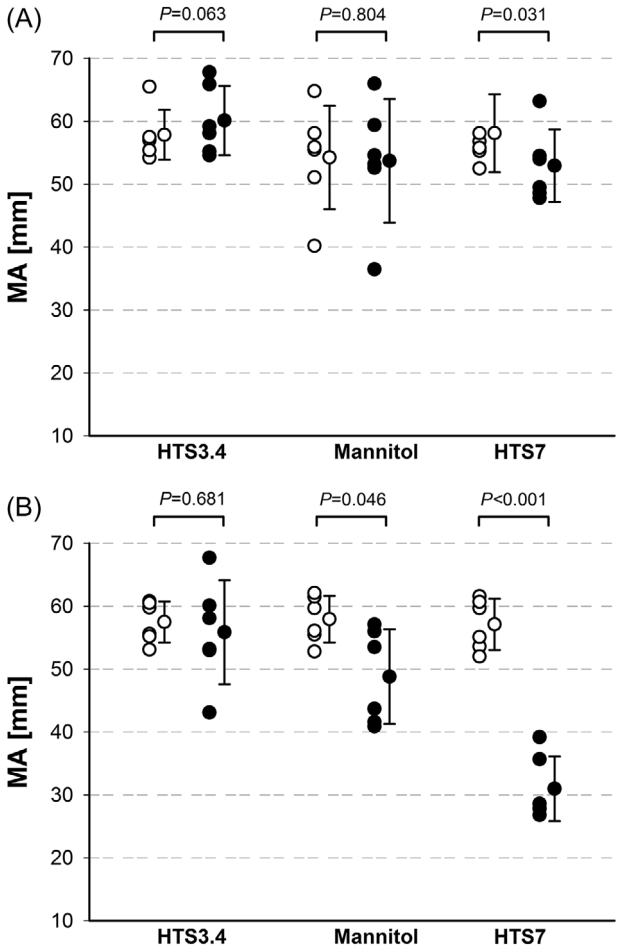

FIGURE 2 (A) Effect of replacing 8% of canine whole blood with intravenous fluids of different osmolarity and tonicity on maximum amplitude (MA). Dots represent individual measurements, bars and whiskers the mean and standard deviation. Open circles, control (undiluted) samples; Solid circles, experimental (diluted) samples. HTS 3.4, dilution with 3.4% hypertonic saline solution; HTS 7, dilution with 7% hypertonic saline solution. $P < 0.05$ was considered statistically significant. (B) Effect of replacing 16% of canine whole blood with intravenous fluids of different osmolarity and tonicity on maximum amplitude (MA). Dots represent individual measurements, bars and whiskers the mean and standard deviation. Open circles, control (undiluted) samples; Solid circles, experimental (diluted) samples. HTS 3.4, dilution with 3.4% hypertonic saline solution; HTS 7, dilution with 7% hypertonic saline solution. $P < 0.05$ was considered statistically significant

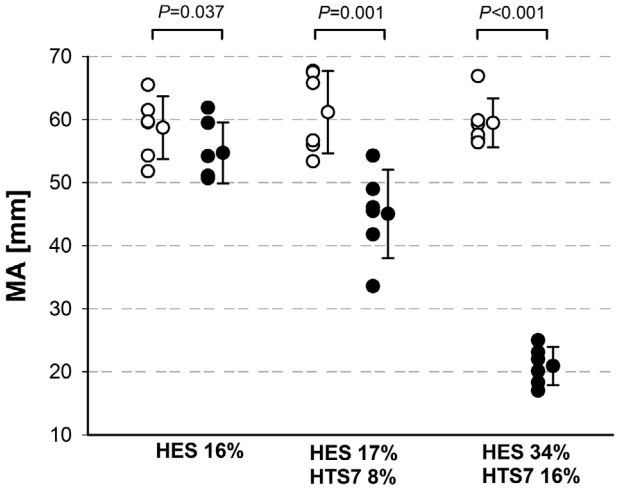

FIGURE 3 Effect of replacing 16% of canine whole blood with hydroxyethyl starch 130/0.4 (HES), or 25% and 50% of whole blood with a mixture of HES and 7% hypertonic saline (HTS 7). Dots represent individual measurements, bars and whiskers, the mean, and SD. Open circles, control (undiluted) samples; Solid circles, experimental (diluted) samples. $P < 0.05$ was considered statistically significant

with the LRS dilution. At 16% dilution, α angle was decreased, while further increasing fluid dilutions to 33% and 66%, not only decreased α angle, but also decreased MA, and prolonged K time at the highest dilution in this model. The incremental effect of blood dilution on coagulation was also found in in vitro studies using human blood, whereby dilution with isotonic crystalloids at 40% or greater consistently led to hypocoagulable results.[29,30] These effects are predominantly a consequence of hemodilution, as clotting factor activities and platelet counts decreased in proportion to dilution.[11]

A 7% hypertonic crystalloid solution has an osmolarity of 2394 mOsm/L and is recommended for management of hemorrhagic shock and intracranial hypertension secondary to trauma.[31] Its benefits are attributed to its hypertonic effects allowing transient fluid shifting from the extravascular into the intravascular space, and its direct impact on the cardiovascular system through vasodilation and improvement in cardiac contractility.[32] In the present study, HTS 7 dilution caused the most severe effects on coagulation at a given dilution in vitro. Unlike the other fluid types examined, HTS 7 dilution affected all TEG variables. Its effects were more pronounced when

TABLE 3 Comparison of hypertonic saline, hydroxyethyl starch, and hypertonic saline/hydroxyethyl starch combination dilutions

			TEG Variable				
Fluid type	**Dilution**	**N**	**R-time (min)**	**K-time (min)**	**α angle (degree)**	**MA (mm)**	**LY60 (%)**
HTS 7	**8%**	6	2.55 (1.18)	1 (0.3)	77.95 (3.9)	48.83 (5.68)	0.55 (0.775)
HES 130/0.4	**16%**	6	1.35 (0.48)	0.85 (1.76)	79.05 (1.6)	52.65 (7.15)	4.65 (1.38)
HTS 7-HES 130/0.4	**25%**	6	3.2 (1.88)[§]	1.65 (0.63)[*,§]	74.05 (7.83)[§]	45.8 (5.55)[*,§]	1.1 (3.1)

HTS 7, 7% hypertonic saline; HES 130/0.4, hydroxyethyl starch 130/0.4; HTS 7-HES 130/0.4, 7% hypertonic saline and hydroxyethyl starch 130/0.4 combination fluid. Data presented as median (inter-quartile ranges).

[*] $P < 0.05$ comparing HTS 7 and HTS 7-HES 130/0.4;

[§] $P < 0.05$ comparing HES 130/0.4 and HTS 7-HES 130/0/4.

compared with LRS at the same dilution, suggestive of a mechanism other than fluid dilution alone. Our results showed that HTS 7 prolonged R and K time, decreased α angle and MA, and increased LY 60. A recently published canine study utilizing a viscoelastic device, thromboelastometry, showed similar findings of slowed dynamics of clot formation and decreased clot strength induced by HTS 7 at 10% dilution, though in contrast to our results, no significant changes were noted in clotting time despite a trend toward prolongation.[22] The differences in our findings may be due to different viscoelastic devices utilized or the higher dilution percentages that were used in our study. Likewise, HTS 7 has also been shown experimentally to affect hemostasis of human blood. At a 5% dilution, platelet aggregation was found to be impaired.[33] At a 10% dilution hypocoagulable traces on TEG were recorded, with the clotting time and dynamics of clot formation being compromised but overall clot strength, unlike to our study, not being diminished.[34] This may reflect interspecies differences with dogs being more vulnerable to alterations from HTS 7 than people. It has been hypothesized that the effects of hypertonic saline on hemostasis may be due to its high tonicity.[35] The tonicity of solutions can impair enzyme function, inactivate clotting factors and hence prolong the clotting time. It may also alter platelet shape that can attenuate the initiation phase of thrombin generation and the activation of platelets altogether.[34,36]

To examine the impact of dilution with hyperosmolar fluids on coagulation further, HTS 3.4 was also examined. It has only half the osmolarity compared to HTS 7 and our findings show that its impact on coagulation by TEG is largely absent at the dilutions examined. Prolonged R time was the only significantly affected TEG variable when compared with equivalent LRS dilution. The less apparent effect of HTS 3.4 highlights the fact that the higher tonicity in HTS 7 constitutes an important role on how this fluid affects hemostasis. It has been demonstrated that hypertonic glycerine in a non-ionic solution iso-osmolar to HTS 7 does not alter coagulation when mixed with blood.[36] The effect of sodium, the major contributor to the hypertonicity of HTS 7, may disturb clotting factor activation and eventually affect platelet and clot strength at higher concentrations or dilutions.[36]

HTS 3.4 was compared to an iso-osmolar non-crystalloid solution (20% mannitol) in this study. Twenty percent mannitol at 16% dilution decreased the α angle, decreased MA, and decreased LY 60 compared with an equivalent dilution of LRS. The effects of 20% mannitol on coagulation were more pronounced than those of HTS 3.4 suggesting that mannitol may affect coagulation independent to its hyper-osmolarity. A recently published canine study compared mannitol and hypertonic saline solutions utilizing thromboelastometry.[24] While direct comparisons cannot be made with our study due to different concentrations and dilution percentages, the authors also reported more severe impairment of coagulation with mannitol compared to an iso-osmolar hypertonic saline solution. In that study, fibrin clot firmness was disturbed with mannitol dilutions, which may suggest that mannitol impacts the fibrin component of the clot in a distinct way. In addition, platelet aggregation was more severely affected by mannitol compared to hypertonic saline.[24] Similar findings were reported in a human study examining an iso-osmolar solution of mannitol and hypertonic saline.[37] Our study also found a hypofibrinolytic effect with mannitol dilutions at 16%, though neither of the mentioned studies published results regarding fibrinolysis, leaving our finding of a hypofibrinolytic effect of mannitol to be confirmed. Future studies utilizing tissue plasminogen activated TEG may be able to investigate this further.

Taking these findings together, it appears that HTS 3.4 has the least effect on coagulation compared to mannitol or HTS 7 in vitro, a finding worth considering when treating traumatic brain injury with increased intracranial pressure in the presence of active bleeding or coagulopathy. The clinical significance of our in vitro findings is to be questioned until replicated in an in vivo setting. In a recently published pilot study, the administration of routine doses of mannitol or HTS 7 in dogs with intracranial hypertension did not impair coagulation on viscoelastic testing or cause clinical hemorrhage; however, platelet dysfunction was seen at 5 minutes for both solutions and lasted up to 60 minutes for HTS 7.[38]

Synthetic colloids such as HES that can be used for fluid resuscitation as colloids are able to expand plasma volume by retention of colloid molecules in the intravascular space.[39] HES 130/0.4 is suggested to have less of an effect on coagulation compared to other HES solutions due to its lower molecular weight and reduced degree of substitution.[40] In our study, HES 130/0.4 decreased α angle, decreased MA, and increased LY60 when compared to controls, but did not cause any differences when compared with LRS at an equivalent dilution, suggesting that the effects on coagulation are largely due to hemodilution. In contrast, another canine study showed that when HES 130/0.4 was compared to saline at a dilution of 25%, significant changes were found in clotting time and clot strength.[22] A systematic review of HES 130/0.4 effects on viscoelastic changes in humans similarly showed that it leads to hypocoagulability in a dose-dependent fashion, and consistently with dilutions of >40% in vitro when compared to equivalent dilutions of isotonic solutions.[41] The absence of statistically significant findings in our study likely reflects the lower dilution dose at 16% and suggests that the coagulation effects of HES 130/0.4 are likely dose related in dogs as well.

The combination of HTS 7-HES 130/0.4 can also be a fluid of choice for resuscitation, as the hypertonic saline effect of rapid expansion of intravascular volume can be prolonged by the oncotic effect of HES.[42] Our findings suggest that the effects of these fluids on coagulation are amplified when used together compared to when used in isolation. This is reflected by significant changes in all TEG variables with the combination fluid compared to either one alone. These effects may partly be due to higher dilution percentage when combined. However, the effects on coagulation of the combined fluids at 25% are significantly more pronounced compared to LRS at 33% dilution. A similarly exacerbated effect on coagulation was previously reported when both fluids were combined compared to individual effects in both people and dogs.[22,33]

Several limitations need to be considered when interpreting the findings of this study. As it is an experimental benchtop study, the normal physiological transvascular shift of fluids after administration intravenously is not accounted for and hence the effects of

hypertonicity or hyper-osmolarity of the tested fluids are likely exaggerated in our in vitro study. In addition, the modulatory role of the vascular endothelium and the clinical context in which resuscitative fluid therapy is administered are not considered but may amplify or diminish the observed effects on coagulation in vivo.[43]

Limitations in study design deserve further consideration. The investigator was not blinded in the study, hence subjecting results to operator bias. Blood draws were not performed uniformly among study subjects, whereby some dogs had multiple draws and some dogs only had one and were not used across all treatments. However, the linear mixed model corrected for the influence of the animal and sample number resulting in adjusted values. Moreover, concurrently run control samples for each dilution processed were utilized to minimize individual effects on results obtained. The effect of repeat sampling from the same animal within a short period of time may have also affected TEG results, as traumatic sampling initiates coagulation, shortening R time.[25] Additionally, the small sample size for each fluid dilution group may have resulted in type II error for some of the results.

Our results demonstrate that in vitro dilution of canine whole blood with commonly used IV fluids leads to thromboelastographic changes consistent with hypocoagulability in a dose dependent manner for all fluid types tested. Next to dilution percentage, viscoelastic changes are influenced by fluid characteristics, specifically tonicity, osmolarity, and colloidal properties. HTS 7, a fluid with high osmolarity and tonicity, exerted the most profound effect on coagulation at the volumes used. This differential effect of fluid characteristics could be taken into consideration when resuscitating dogs with large fluid volumes, but clinical studies are required to further delineate the importance of different resuscitation fluids and volumes on hemostasis in dogs.

ENDNOTES

* Vacuette Tube 1 mL 9NC Coagulation sodium citrate 3.2%, Grenier Bio-one, West Heidelberg, VIC, Australia.

† MiniCollect Tube 1 mL K3E K3EDTA, Grenier Bio-one, West Heidelberg, VIC, Australia.

‡ Sysmex XT 2000i Automated Hematology Analyzer, Sysmex, Macquarie Park, NSW, Australia.

§ MiniCollect Lithium Heparin Tube, Grenier Bio-one, West Heidelberg, VIC, Australia.

¶ Cobas Integra 400 plus, Roche Products Pty Ltd, Sydney, NSW, Australia.

7% NaCl, Sykes Vet International Pty Ltd, Dandenong South, VIC, Australia.

‖ 7% NaCl, Sykes Vet International Pty Ltd, Dandenong South, VIC, Australia.

** 20% Osmitrol, Baxter Healthcare Pty Ltd, Old Toongabie, NSW, Australia.

†† Compound sodium lactate (Hartmann's solution), Baxter Healthcare Pty Ltd, Old Toongabie, NSW, Australia.

‡‡ Voluven 6%, Fresnius Kabi, Freidberg, Germany.

§§ 2-channel 5000 series TEG analyzer, Hemoscope Corporation, Niles, IL.

¶¶ Kaolin, Haemonetics, North Ryde, NSW, Australia.

Disposable Cups and Pins, Haemonetics, North Ryde, NSW, Australia.

‖‖ Microsoft Excel, Microsoft Pty Ltd, North Ryde, NSW, Australia.

*** Allegra 6R Centrifuge, Beckman Coulter, Lane Cove, NSW, Australia.

††† Stago STA Compact Max analyzer, Diagnostica Stago, Doncaster, VIC, Australia.

‡‡‡ STA-Liquid FIB, Diagnostica Stago, Doncaster, VIC, Australia.

§§§ Genstat 16th Edition, VSNi International Ltd, Hemel Hempstead, UK.

¶¶¶ JMP 12, SAS Institute, Cary, NC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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