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STANDARD ARTICLE

Effects of 6% tetrastarch or lactated Ringer's solution on blood coagulation in hemorrhaged dogs

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Background: Tetrastarch solution (TS) can impair coagulation but the clinical relevance of this impairment is unclear in veterinary medicine.

Objective: Compare the effects of volume replacement (VR) with lactated Ringer's solution (LRS) or 6% TS on coagulation in hemorrhaged dogs.

Animals: Six healthy English Pointer dogs (19.7-35.3 kg).

Methods: Prospective crossover study. Dogs were anesthetized without hemorrhage and VR (control). Two weeks later, dogs were hemorrhaged under anesthesia on 2 occasions (8-week washout intervals) and randomly received VR with LRS or TS at 3:1 or 1:1 of shed blood, respectively, aiming to decrease the hematocrit to 33%. Rotational thromboelastometry and other coagulation variables were determined before 0.5, 2, and 4 hours after VR during anesthesia and 24 hours after VR (conscious dogs).

Results: Buccal mucosal bleeding time did not differ between treatments after VR. Activated partial thromboplastin time increased from controls 4 hours after TS ($P = 0.045$). Clot formation time (CFT) and alfa-angle increased from controls from 0.5 to 4 hours after LRS (CFT, $P \leq 0.0001$-0.02; alpha angle, $P = 0.0001$-0.02) and from 0.5 to 2 hours after TS (CFT, $P = 0.0002$-0.01; alpha angle, $P = 0.0005$-0.02). The maximum clot firmness decreased from controls from 0.5 to 4 hours after LRS ($P \leq 0.0001$-0.01) and TS ($P \leq 0.0001$-0.04).

Conclusions and Clinical Relevance: Tetrastarch does not impair primary hemostasis and induces transient dilutional coagulopathy that is similar to LRS because, when compared to a 3 times higher volume of LRS in hemorrhaged dogs, it does not cause greater interference on the viscoelastic properties of the coagulum.

KEYWORDS

coagulation, colloids, crystalloids, hydroxyethyl starch, thromboelastometry

1 | INTRODUCTION

Use of hydroxyethyl starch (HES) solutions is controversial in human medicine because the use of these fluids may be associated not only with acute kidney injury but also with impaired coagulation.[1,2] The coagulopathy associated with HES solutions may involve several mechanisms, including: (1) inhibition of the formation of the fibrin network because of binding and inactivation of factor VIII and von

Abbreviations: Alpha-angle$_X$, alpha-angle "x" indicating "activation by the extrinsic (ex-TEM) or intrinsic (in-TEM) coagulation pathways; **aPTT**, activated partial thromboplastin time; **BMBT**, buccal mucosal bleeding time; **CFT$_X$**, clot formation time, "x" indicating activation by the extrinsic (ex-TEM) or intrinsic (in-TEM) coagulation pathways; **CT$_X$**, clotting time, "x" indicating activation by the extrinsic (ex-TEM) or intrinsic (in-TEM) coagulation pathways; **HES**, hydroxyethyl starch; **LRS**, lactated Ringer's solution; **MCF$_X$**, maximum clot firmness, "x" indicating activation by the extrinsic (ex-TEM) or intrinsic (in-TEM) coagulation pathways; **PT**, prothrombin time; **ROTEM**, rotational thromboelastometry; **TS**, 6% tetrastarch solution; **vWF**, von Willebrand's factor

Willebrand's factor (vWF); (2) inhibition of blood clot formation by inhibition of glycoproteins GP IIb/IIIa located on the surface of activated platelets; (3) inhibition of the binding of GP IIb/IIIa platelet receptors to vWf and fibrinogen; and (4) acceleration of fibrin degradation.[2–4]

Hydroxyethyl starch solutions with smaller molecular weight and lower molar substitution (percentage of glucose molecules chemically modified by the addition of hydroxyethyl groups) are more rapidly metabolized and eliminated with an apparent decrease in some adverse effects.[1,4] Tetrastarch solution (TS) is a third generation HES that has a lower molecular weight (130 kDa) and molar substitution (0.4) than other HES solutions (hetastarch [450 kDa/0.7] and pentastarch [200 kDa/0.5]). It has been speculated that dogs breakdown and eliminate HES solutions more rapidly than humans because of a higher activity of plasma alpha amylase.[5] Because TS is more rapidly cleared from the circulation than previous generations of HES, direct inhibition of factor VIII and vWF could be minimized and the negative effects of this colloid on coagulation might be lessened.[6]

Tetrastarch may induce a transient hypocoagulable state in dogs.[7,8] Results of a study showed that a large volume of TS (40 mL/kg), compared to an equal volume of physiological saline, impaired coagulation in healthy and endotoxemic dogs.[7] In anesthetized normovolemic dogs, a 15 mL/kg bolus of TS diluted in either physiological saline or balanced electrolyte solution transiently impaired primary (evaluated by a platelet function analyzer) and secondary (assessed by rotational thromboelastometry [ROTEM]) hemostasis in relation to the same volume of physiological saline.[8] The authors concluded that the coagulopathy induced by TS was short-lived, but the duration of these effects could not be fully clarified because hemostasis was assessed 5 minutes and 3 hours after fluid administration.[8] Although these studies showed that coagulation can be impaired by TS, the clinical application of these findings is not clear because TS was compared to equal volumes of isotonic crystalloids. The volume expansion efficiency (ratio of plasma volume expansion to the volume of infused fluid) of TS far exceeds that achieved by isotonic crystalloids.[9,10] Therefore, TS likely will cause dilutional coagulopathy if compared to equal volumes of isotonic crystalloids. The recommended volume of isotonic crystalloids for volume replacement (VR) after acute hemorrhage is at least 3 times the volume of shed blood.[9,10] Because the volume expansion efficiency of TS is approximately 1:1, the volume of shed blood can be replaced by the same volume of TS.[9,10]

Our study aimed to compare the effects of VR with lactated Ringer's solution (LRS) or TS, administered at a ratio of 3:1 and 1:1 of shed blood, respectively, on coagulation (primary and secondary hemostasis) in hemorrhaged dogs.

2 | MATERIAL AND METHODS

2.1 | Animals

Previous approval by the Institutional Animal Care Committee was obtained under protocol number 43/2015. Six healthy purpose-bred English Pointer dogs (weight range, 19.4-35.8 kg), 4 males and 2 females, 47-48 months old, were used. All animals were considered healthy based on physical and laboratory investigations (CBC, serum biochemistry, urinalysis, venous blood gas analysis and electrolytes) that were within normal ranges. After the end of the study, all animals were adopted by private owners.

2.2 | Study design

This prospective, crossover, opportunistic study was carried out in conjunction with another study that aimed to evaluate the effects of VR with LRS or TS after hemorrhage on extravascular lung water and markers of acute kidney injury.[11] During phase 1, animals were anesthetized for data collection without any intervention (control treatment). After a 2-week washout interval, 2 other anesthetic episodes were carried out on the same animals (8-week washout intervals). During each anesthetic episode, dogs were hemorrhaged and randomly assigned[12] to receive VR or LRS or TS at ratios of 3:1 and 1:1 of the amount of shed blood (LRS and TS treatments; phase 2).

2.3 | Experimental protocol and variables monitored

After being fasted for 12 hours, animals received 0.5 mg/kg of morphine (Sulfato de morfina 10 mg/mL, Cristália, Itapira, Brazil) IM and anesthesia was induced with isoflurane (Isoforine®, Cristália, Itapira, Brazil). After an endotracheal tube was placed, anesthesia was maintained with isoflurane and a constant rate infusion of remifentanil hydrochloride (Ultiva®, GlaxoSmithKline, Rio de Janeiro, Brazil) (0.15 μg/kg/min) administered concomitantly with a LRS infusion (2 mL/kg/hour) by means of a 20-gauge catheter (Insyte, Becton Dickinson, Juiz de Fora, Brazil) placed in the cephalic vein.

After induction of anesthesia, an 18-gauge, 20 cm long, single lumen central venous catheter (Venoseld, Rehlinger-Siersburg, Germany) was aseptically placed into the jugular vein. This catheter was used for collecting blood samples used in coagulation assays and for replacing the shed blood with LRS or TS. Another 7 cm long, 4-French, thermodilution catheter (PiCCO Catheter PV2013L07N, Pulsion Medical Systems, Munich, Germany) was inserted into the femoral artery for collecting samples for blood gas analysis and for measuring mean arterial pressure and cardiac output for a parallel study.[11]

Depth of anesthesia was adjusted by changes in end-tidal isoflurane concentrations (Dräger Primus, Drägerwerk AG & Co, Lübeck, Germany), which were adjusted to prevent movement and to maintain mean arterial pressure between 60 and 70 mm Hg throughout anesthesia.[11] Conditions of normocapnia ($PaCO_2$ between 35 and 45 mm Hg) were achieved during anesthesia by use of mechanical ventilation (Dräger Primus, Drägerwerk AG & Co, Lübeck, Germany). Esophageal temperature was maintained between 37.5°C and 38.5°C by means of a forced warm air device (Bair Hugger, Arizant Healthcare, Mineapolis, MN).

After a 2-hour equilibration period, hematocrit, platelet count, buccal mucosal bleeding time (BMBT), prothrombin time (PT), activated partial thromboplastin time (aPTT), and ROTEM assays were performed at baseline (BL). After BL, animals were hemorrhaged. The volume of shed blood was determined using a formula that estimates the volume of blood that could theoretically be withdrawn from an

individual to decrease the hematocrit to a predefined target value after VR.[13] The amount of shed blood was calculated with the aim to decrease the hematocrit to 33%. This blood volume was removed during 30 minutes from a 20-gauge catheter placed in the dorsal pedal artery into collection bags containing the proper amount of sodium citrate, phosphate, dextrose, and adenine (CPDA blood collection bag, JP Indústria Farmacêutica SA, Ribeirão Preto, Brazil). The bags were weighted during blood removal to control the exact volume of shed blood. Immediately after the calculated amount of blood was removed, VR with LRS or TS was performed over 30 minutes at a ratio of 3:1 and 1:1, respectively. Coagulation variables were recorded at 0.5, 2, and 4 hours after VR with LRS or TS. After data collection at the 4-hour time point, the femoral and cephalic catheters were removed, and anesthesia was stopped. All variables were recorded again 24 hours after VR in conscious animals and the central venous catheter was removed immediately after blood collection.[11]

2.4 | Hematocrit, platelet count, BMBT, PT, and aPTT

Blood samples were placed in heparinized microhematocrit tubes and centrifuged (Micro Hematócrito - MH, Celm, São Caetano do Sul, Brazil) at 11 500 rpm for 5 minutes for measuring hematocrit. Blood samples with EDTA were used for platelet count, which was carried out manually using a Neubauer chamber.

A spring-loaded template device (Triplett Bleeding Time Test Device, Helena Laboratories, Beaumont, TX) was applied by a single individual to the everted upper lip to create a 1 mm deep and 5 mm long incision in the mucosa. The bleeding from the everted upper lip was absorbed by a filter paper, without touching the wound, and the time elapsed from creating the incision until the bleeding stopped was recorded as the BMBT. Prothrombin time and aPTT were measured in citrated blood using a point-of-care coagulation analyzer (Coag Dx Analyzer, IDDEX, Westbrook, ME).

2.5 | Rotational thromboelastometry

The viscoelastic properties of the blood clot were evaluated by ROTEM (ROTEM delta, Tem International GmbH, Munich, Germany) analysis. Citrated blood (300 μL) samples were added to 2 cuvettes. After blood samples were recalcified with 20 μL of calcium chloride (0.2 M), the extrinsic (ex-TEM) and intrinsic (in-TEM) coagulation pathways were activated by adding human recombinant tissue factor (ex-TEM, Tem International GmbH, Munich, Germany) and partial thromboplastin phospholipid/ellagic acid (in-TEM, Tem International GmbH Munich, Germany) to each cuvette, respectively. Coagulation was allowed to proceed for 60 minutes to provide values of clotting time (CT, the time elapsed from coagulation pathway activation and initial fibrin formation), clot formation time (CFT, the time from CT until a clot firmness of 20 mm is achieved), alpha-angle (the angle between CT and CFT points, a measure of the speed of clot formation), and maximum clot firmness (MCF, a measure of the maximum resistance or firmness of the clot).

2.6 | Data analysis

Using commercial statistical software (Prism 6.02, GraphPad, San Diego, CA), normality of data distribution was verified by a Kolmogorov-Smirnov test. Buccal mucosal bleeding time, PT, aPTT, and $CT_{ex\text{-}TEM}$ were asymmetrically distributed and were log transformed before analysis. A paired *t* test was used to compare the total

TABLE 1 Hematocrit, platelet count, buccal mucosal bleeding time (BMBT), prothrombin time (PT), activated partial thromboplastin time (aPTT). Data represent the mean ± SD of 6 dogs that did not undergo acute hemorrhage followed by volume replacement (VR) in one occasion (control treatment) or that underwent hemorrhage and randomly received VR with lactated Ringer's solution (LRS treatment) or with 6% tetrastarch solution (TS treatment) at a ratio of 3:1 or 1:1 the volume of shed blood, respectively. Variables were recorded during anesthesia, before hemorrhage (BL), 0.5, 2, and 4 hours after VR. Anesthesia was interrupted 4 hours after VR, and data were collected 24 hours after VR

				Time after VR, hours			
Variable	Reference range	Treatment	BL	0.5	2	4	24
Hematocrit, %	38-48	Control	43 ± 3	41 ± 4[a]	40 ± 2[a]	40 ± 2[a]	45 ± 3[a]
		LRS	41 ± 2	32 ± 2[b]	34 ± 2[b]	37 ± 3[b]	36 ± 3[b]
		TS	42 ± 3	32 ± 4[b]	35 ± 2[b]	36 ± 1[b]	39 ± 3[c]
Platelet count, $\times 10^3/\mu L$	200-500	Control	332 ± 44	346 ± 67[a]	327 ± 36[a]	317 ± 48[a]	314 ± 43[a]
		LRS	323 ± 57	213 ± 47[b]	262 ± 61[b]	252 ± 67[b]	242 ± 35[b]
		TS	302 ± 42	232 ± 28[b]	291 ± 54[ab]	294 ± 40[ab]	268 ± 16[b]
BMBT, second	<240	Control	61 ± 16[ab]	63 ± 15	57 ± 14	56 ± 12	97 ± 40
		LRS	86 ± 22[a]	56 ± 14	60 ± 19	52 ± 15	80 ± 38
		TS	50 ± 16[b]	74 ± 21	79 ± 35	51 ± 21	76 ± 39
PT, second	11-17	Control	15 ± 1	15 ± 1[a]	17 ± 3	15 ± 2	13 ± 1
		LRS	14 ± 1	16 ± 2[a]	15 ± 1	15 ± 1	13 ± 1
		TS	14 ± 1	13 ± 1[b]	16 ± 1	14 ± 1	13 ± 1
aPTT, second	72-102	Control	72 ± 9	73 ± 9	75 ± 14	73 ± 9[a]	80 ± 11
		LRS	75 ± 12	92 ± 29	82 ± 15	83 ± 9[ab]	87 ± 6
		TS	79 ± 15	89 ± 24	90 ± 14	91 ± 16[b]	79 ± 10

Within each column. For a given time point, treatment group means followed by different superscript letters are significantly different from each other (Tukey's test, $P \leq 0.05$).

amount of blood withdrawn in the LRS and TS treatment groups. Comparisons among treatment groups were performed by a 2-way ANOVA for repeated measures by both factors (time and treatment) followed by post hoc comparisons among treatment groups using a Tukey's test adjusted for the repeated measure design. For all variables, the significance level was set at $P < 0.05$.

3 | RESULTS

3.1 | Effects of LRS and TS on hematocrit, platelet count, BMBT, PT, and aPTT (Table 1)

The total volume of blood removed did not differ between LRS and TS treatments (25 ± 7 and 23 ± 4 mL/kg, respectively). The volumes of LRS and TS infused for VR were 75 ± 14 and 23 ± 4 mL/kg ($P \leq 0.0001$).

The hematocrit decreased in comparison to controls from 0.5 to 24 hours after VR with LRS ($P \leq 0.0001$-0.02) and TS ($P \leq 0.0001$-0.001; Table 1). Hematocrit was significantly higher 24 hours after VR with TS than with LRS ($P = 0.02$).

Platelet count significantly decreased from controls from 0.5 to 24 hours after LRS ($P \leq 0.0001$-0.002) and at 0.5 and 24 hours after TS administration ($P \leq 0.0001$-0.03). Except for 1 dog, in which transient mild thrombocytopenia was observed from 0.5 to 4 hours (126-172 × 10^3/μL) after VR with LRS, platelet count remained within normal ranges in all treatment groups.

Buccal mucosal bleeding time recorded for individual animals remained within normal ranges throughout the study in all treatment groups. A significant difference in BMBT was observed between LRS and TS treatments at BL ($P = 0.005$), but values recorded in these treatment groups did not differ significantly from controls at BL. For the remainder of the observational period, BMBT did not differ significantly among treatments.

At 0.5 hours after VR, PT decreased significantly in the TS treatment group compared to control ($P = 0.04$) and LRS treatments ($P = 0.007$). At 4 hours after VR, aPTT was significantly increased in the TS treatment in comparison to controls ($P = 0.045$). Mean PT and aPTT values remained within normal reference ranges provided by the equipment manufacturer throughout the study. An increase in aPTT above the reference range was recorded 0.5 hours after VR with LRS

TABLE 2 Rotational thromboelastometry-derived variables of clotting time (CT), clot formation time (CFT), alpha-angle, and maximum clot firmness (MCF) measured from citrated blood in which coagulation was activated by the extrinsic (ex-TEM) and intrinsic pathways (in-TEM). Data represent the mean ± SD of 6 dogs that did not undergo acute hemorrhage followed by VR in one occasion (control) or that underwent hemorrhage and randomly received VR with lactated Ringer's solution (LRS treatment) or with 6% TS treatment at a ratio of 3:1 or 1:1 the volume of shed blood, respectively. Variables were recorded during anesthesia, before hemorrhage (BL), 0.5, 2, and 4 hours after VR. Anesthesia was interrupted 4 hours after VR, and data were collected 24 hours after VR

				Time after VR, hours			
Variable	Reference range[14]	Treatment	BL	0.5	2	4	24
$CT_{ex\text{-}TEM}$, second	29-92	Control	40 ± 10	37 ± 2	43 ± 8	41 ± 12	35 ± 7
		LRS	49 ± 17	57 ± 30	54 ± 16	56 ± 18	34 ± 14
		TS	44 ± 16	63 ± 14	47 ± 8	48 ± 18	48 ± 17
$CFT_{ex\text{-}TEM}$, second	54-275	Control	96 ± 12	96 ± 10^a	89 ± 14^a	92 ± 14^a	90 ± 11
		LRS	106 ± 12	129 ± 43^b	118 ± 25^b	113 ± 24^b	100 ± 17
		TS	95 ± 8	116 ± 22^b	111 ± 18^b	104 ± 16^{ab}	91 ± 12
Alpha-$angle_{ex\text{-}TEM}$, °	47-79	Control	70.8 ± 2.3	70.7 ± 2.0^a	72.2 ± 2.5^a	71.5 ± 2.8^a	72.3 ± 2.2
		LRS	69.8 ± 2.9	67.3 ± 2.8^b	67.2 ± 3.3^b	67.8 ± 4.2^b	71.3 ± 1.9
		TS	71.5 ± 1.6	67.7 ± 3.3^b	68.3 ± 2.7^b	69.5 ± 2.9^{ab}	72.2 ± 2.2
$MCF_{ex\text{-}TEM}$, mm	36-73	Control	62.0 ± 3.4^a	62.7 ± 3.3^a	61.7 ± 4.3^a	62.2 ± 3.6^a	66.2 ± 3.3
		LRS	57.8 ± 4.7^b	52.5 ± 5.2^b	53.7 ± 5.8^b	54.8 ± 5.7^b	64.2 ± 2.9
		TS	60.8 ± 3.4^{ab}	54.7 ± 3.5^b	56.7 ± 3.1^b	57.5 ± 3.9^b	63.8 ± 4.4
$CT_{in\text{-}TEM}$, second	126-363	Control	116 ± 23	109 ± 16	105 ± 17	113 ± 11	105 ± 33
		LRS	120 ± 21	105 ± 41	108 ± 17	115 ± 18	121 ± 30
		TS	106 ± 14	97 ± 29	93 ± 23	109 ± 25	106 ± 33
$CFT_{in\text{-}TEM}$, second	47-224	Control	76 ± 13	80 ± 15^a	75 ± 13^a	84 ± 23^a	76 ± 13
		LRS	93 ± 16	133 ± 46^b	116 ± 32^b	109 ± 23^b	82 ± 15
		TS	77 ± 16	116 ± 32^b	112 ± 18^b	100 ± 20^{ab}	81 ± 11
Alpha-$angle_{in\text{-}TEM}$, °	51-81	Control	76.2 ± 2.3	74.8 ± 2.4^a	75.5 ± 2.4^a	75.2 ± 2.5	76.5 ± 4.5
		LRS	73.3 ± 2.7	69.7 ± 6.9^b	70 ± 3.6^b	71 ± 3.2	73.8 ± 2.6
		TS	75.8 ± 3.3	70.5 ± 2.8^b	69.5 ± 4.1^b	72.8 ± 2.9	74 ± 2.1
$MCF_{in\text{-}TEM}$, mm	50-75	Control	60.2 ± 3.2	61 ± 4.1^a	61.2 ± 3.1^a	60 ± 4.8^a	64.3 ± 3.3
		LRS	55.5 ± 3.8	51.8 ± 4.5^b	52.5 ± 3.9^b	54.2 ± 3.3^b	59.8 ± 7.3
		TS	58.5 ± 2.7	55.7 ± 3.4^b	54.3 ± 2.6^b	55.2 ± 4.7^b	62 ± 4

Within each column. For a given time point, treatment group means followed by different superscript letters are significantly different from each other (Tukey's test, $P \leq 0.05$).

in 2/6 dogs (103 and 140 seconds). Individual aPTT values were above the reference range in 3/6 dogs after VR with TS. In 1 dog, aPTT increased from 80 seconds (BL) to 124, 116, and 115 seconds at 0.5, 2, and 4 hours after TS, respectively, returning to normal 24 hours after VR (86 seconds). In the 2 remaining dogs, aPTT was above the reference range at 1 time point after TS administration (114 and 106 seconds in each animal, respectively).

3.2 | Effects of LRS and TS on ROTEM assays (Table 2)

The $CFT_{ex\text{-}TEM}$ and $CFT_{in\text{-}TEM}$ were significantly higher than controls from 0.5 to 4 hours after LRS ($P \leq 0.0001$-0.01) and from 0.5 to 2 hours after TS ($P = 0.0002$-0.01). The $\alpha\text{-angle}_{ex\text{-}TEM}$ and $\alpha\text{-angle}_{in\text{-}TEM}$ were significantly lower than controls from 0.5 to 2 hours after LRS ($P = 0.0001$-0.02) and TS ($P = 0.0005$-0.02). At 4 hours after VR, the $\alpha\text{-angle}_{ex\text{-}TEM}$ was lower than controls only in the LRS treatment ($P = 0.004$). The $MCF_{ex\text{-}TEM}$ and $MCF_{in\text{-}TEM}$ values were significantly lower than controls from 0.5 to 4 hours after VR with LRS ($P \leq 0.0001$-0.01) and TS ($P \leq 0.0001$-0.04).

Rotational thromboelastography derived parameters (mean values) were within reference ranges reported in the literature,[14] except for mean $CT_{in\text{-}TEM}$ values that were lower than reference ranges throughout the observational period in all treatment groups.

4 | DISCUSSION

Use of TS for VR after hemorrhage resulted in dilutional coagulopathy because the hypocoagulable state induced by the colloid was equivalent or less than that induced by a 3 times higher volume of LRS. The increase in the time of clot formation (CFT) induced by VR with TS was of shorter duration in comparison to VR with LRS (2 and 4 hours, respectively). However, the decrease in clot strength (MCF) was of similar magnitude and duration for both fluids (4 hours). Because TS did not cause more interference with the viscoelastic properties of the blood clot in comparison to LRS, a fluid known for not causing direct inhibition of coagulation, TS probably did not cause a significant inhibition of coagulation factors.

Changes in ROTEM assays induced by VR with LRS or TS were compared with control values previously generated using the same animals undergoing the same experimental protocol without hemorrhage and VR. This design has the advantage of avoiding bias caused by individual responses on the interventions under evaluation. Although VR with TS and LRS decreased the speed of clot formation and the firmness of the blood clot, with the exception of mean $CT_{in\text{-}TEM}$ values, the mean values derived from ROTEM assays remained within the reference intervals previously published by another laboratory.[14] These findings suggest that TS may be used for VR in healthy dogs without causing a clinically relevant increase in the risk of bleeding.

Based on a pilot study, the hemorrhage model targeting a decrease in hematocrit to 33% would allow removal of approximately 30% of blood volume (24 mL/kg, considering the blood volume as 80 mL/kg). In a previous study of dogs anesthetized using a fixed end-tidal isoflurane concentration (1.3 times the minimum alveolar concentration), removal of 30% of blood volume resulted in hypotension (MAP < 60 mm Hg) in half of the studied population (4/8 dogs).[15] Because of concerns with renal injury in animals that would receive a fluid that might predispose to renal damage (TS),[2,4] the end-tidal isoflurane concentration was adjusted to provide immobility and to maintain MAP within ranges that usually are considered acceptable during anesthesia (MAP between 60 and 70 mm Hg).[11]

Differences in the degree of dilution of coagulation factors may be an important confounder in studies comparing the effects of IV fluids on blood coagulation. In dogs with hypotensive hemorrhagic shock, the volume expansion efficiency (effective plasma volume expansion divided by the infused volume) is rapidly decreased from 38% at 5 minutes after LRS administration to 11% approximately 90 minutes later.[9] In contrast, the volume expansion efficiency of TS is close to 100% during the same time period.[9] Based on a previous report,[9] the ratio between the volume of LRS administered and the blood volume removed (3:1) could not have fully compensated for the redistribution of this fluid from the intravascular to the extravascular space. However, similar decreases in hematocrit induced by both fluids suggest that a similar degree of hemodilution was achieved when VR was performed with LRS or TS. On the other hand, the decrease in platelet count induced only by LRS in comparison to controls at 2 and 4 hours after VR might suggest that the degree of hemodilution could have been greater for LRS than for TS at these time points. This difference in platelet count, however, probably did not interfere with primary hemostasis because no significant differences in BMBT were found among treatments after VR, and the mean platelet count remained within the normal range for the dogs.

In vitro models have been used to evaluate the effects of colloids on platelet function and on ROTEM in canine blood.[14,16] However, comparisons between LRS and TS using in vitro assays have limited clinical application because this model cannot account for the redistribution of fluids that occurs from the intravascular to the extravascular space. In addition, the magnitude and duration of the effects of colloids on hemostasis can altered by the metabolism of colloids in the circulation,[1] a situation that cannot be mimicked during in vitro conditions.

Hydroxyethyl starch solutions may interfere with vWF activity and concentration, which in turn may impair platelet aggregation (primary hemostasis).[4,17] A large volume of TS (40 mL/kg) decreased vWF concentration in comparison to the same volume of physiological saline in dogs, suggesting that primary hemostasis may be decreased by the colloid.[7] However, recent studies have shown that TS administration after hemorrhagic shock does not alter primary hemostasis (ie, platelet function) beyond a dilutional effect in dogs.[18] Contrasting with large molecular weight HES solutions, TS has little impact on platelet aggregation in dogs and humans.[16,18,19] Although the activity of vWF and platelet aggregation were not evaluated in our study, primary hemostasis was clinically unaffected by either fluid because BMBT, which qualitatively evaluates platelet function (ie, ability to create an effective platelet plug), did not differ between the LRS and TS groups, and remained within reference values for the species.

An increase in aPTT with normal PT might reflect a defect in factor VIII, other intrinsic coagulation pathway factors (XII, XI, and IX), or both. Activated partial thromboplastin time transiently increased from controls 4 hours after TS administration ($P = 0.045$), suggesting that TS caused an impairment in the intrinsic coagulation pathway. Hydroxyethyl starch solutions may interfere with factor VIII and inhibit the intrinsic coagulation pathway by 2 mechanisms: (1) direct decrease of activated factor VIII beyond a dilutional effect, and (2) direct binding and inactivation of factor VIII.[2,20] Although a decrease in factor VIII activity may be observed with HES solutions of larger molecular weight,[20] large doses of TS administered to dogs (40 mL/kg)[7] and humans (50-70 mL/kg)[21,22] did not decrease factor VIII concentrations. Despite a transient increase in aPTT induced by TS, the decrease in $MCF_{in\text{-}TEM}$ of similar magnitude and duration (4 hours) induced by VR with LRS and TS supports the hypothesis that the hypocoagulable effect of TS was a dilutional effect, and suggests that the intrinsic coagulation pathway (factor VIII) was impaired to the same degree by both fluids. This finding is in agreement with the concept that HES solutions with smaller molecular weight and molar substitution, such as third generation TS, cause less interference with coagulation than HES solutions of larger molecular weight.[1,2,6,23]

The small number of animals in our study raises the concern that there could have been a lack of power to show more subtle differences between treatments. Because of the opportunistic nature of the present study, which was carried out in conjunction with another trial, a sample size of 6 animals was estimated based on expected differences in extravascular lung water.[11] Another shortcoming of the study reported here is the fact that not all variables were similar between treatment groups before hemorrhage. Baseline $MCF_{ex\text{-}TEM}$ was significantly lower in the LRS treatment group compared to controls (62 ± 2.4 versus 57.8 ± 4.7 mm, $P = 0.01$), and this difference could have biased subsequent comparisons between the LRS treatment and the other treatment groups. Although this difference could suggest that the washout intervals between experiments that involved hemorrhage and VR (8 weeks) might not have been long enough to completely eliminate a carryover effect, this interval far exceeds the period required by the bone marrow to restore the depleted cells after blood donation in dogs (10 days).[24] The difference in $MCT_{ex\text{-}TEM}$ was relatively small and might have been caused by type II error.

5 | CONCLUSIONS

The use of TS for VR after hemorrhage in healthy dogs does not appear to impair primary hemostasis. The hypocoagulable state induced by TS was transient (2-4 hours) and, when compared with a 3 times higher volume of LRS, VR with TS at a 1:1 ratio does not cause more interference on the viscoelastic properties of the blood clot in hemorrhaged dogs. These findings suggest that TS induces dilutional coagulopathy, and when used for VR in hemorrhaged dogs, does not increase the risk of bleeding in comparison to isotonic crystalloids.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

This research involved a colony of purpose bred research dogs. The IACUC of the Universidade Estadual Paulista approved this experiment and the animals were donated to private owners after the end of the study.

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REFERENCES

1. Westphal M, James MF, Kozek-Langenecker S, et al. Hydroxyethyl starches: different products--different effects. *Anesthesiology*. 2009; 111:187-202.
2. Toyoda D, Shinoda S, Kotake Y. Pros and cons of tetrastarch solution for critically ill patients. *J Intensive Care*. 2014;25(2):23.
3. Fenger-Eriksen C, Tønnesen E, Ingerslev J, et al. Mechanisms of hydroxyethyl starch-induced dilutional coagulopathy. *J Thromb Haemost*. 2009;7:1099-1105.
4. Glover PA, Rudloff E, Kirby R. Hydroxyethyl starch: a review of pharmacokinetics, pharmacodynamics, current products, and potential clinical risks, benefits, and use. *J Vet Emerg Crit Care*. 2014;24:642-661.
5. Yacobi A, Gibson TP, McEntegart CM, et al. Pharmacokinetics of high molecular weight hydroxyethyl starch in dogs. *Res Commun Chem Pathol Pharmacol*. 1982;36:199-204.
6. Jungheinrich C, Neff TA. Pharmacokinetics of hydroxyethyl starch. *Clin Pharmacokinet*. 2005;44:681-699.
7. Gauthier V, Holowaychuk MK, Kerr CL, Bersenas AME, Darren Wood R. Effect of synthetic colloid administration on coagulation in healthy dogs and dogs with systemic inflammation. *J Vet Intern Med*. 2015;29:276-285.
8. Reuteler A, Axiak-Flammer S, Howard J, et al. Comparison of the effects of a balanced crystalloid-based and a saline-based tetrastarch solution on canine whole blood coagulation and platelet function. *J Vet Intern Med*. 2015;29:276-285.
9. Barros JM, do Nascimento P Jr, Marinello JL, et al. The effects of 6% hydroxyethyl starch-hypertonic saline in resuscitation of dogs with hemorrhagic shock. *Anesth Analg*. 2011;112:395-404.
10. Miller TE, Roche AM, Mythen M. Fluid management and goal-directed therapy as an adjunct to Enhanced Recovery After Surgery (ERAS). *Can J Anaesth*. 2015;62:158-168.
11. Diniz MS, Teixeira-Neto FJ, Celeita-Rodríguez N, et al. Effects of 6% tetrastarch and lactated Ringer's solution on extravascular lung water and

markers of acute renal injury in hemorrhaged, isoflurane-anesthetized healthy dogs. *J Vet Intern Med*. 2018;32:712-721.
12. Urbaniak GC, Plous S, Research Randomizer (Version 4.0) [Computer software]. http://www.randomizer.org/. Accessed June 22, 2013.
13. Gross JB. Estimating allowable blood loss: corrected for dilution. *Anesthesiology*. 1983;58:277-280.
14. Falco S, Bruno B, Maurella C, et al. In vitro evaluation of canine hemostasis following dilution with hydroxyethyl starch (130/0.4) via thromboelastometry. *J Vet Emerg Crit Care (San Antonio)*. 2012; 22:640-645.
15. Diniz MS, Teixeira-Neto FJ, Cândido TD, et al. Effects of dexmedetomidine on pulse pressure variation changes induced by hemorrhage followed by volume replacement in isoflurane-anesthetized dogs. *J Vet Emerg Crit Care (San Antonio)*. 2014;24:681-692.
16. 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.
17. Kozek-Langenecker SA. Effects of hydroxyethyl starch solutions on hemostasis. *Anesthesiology*. 2005;103:654-660.
18. McBride D, Hosgood G, Raisis A. Platelet closure time in anesthetized Greyhounds with hemorrhagic shock treated with hydroxyethyl starch 130/0.4 or 0.9% sodium chloride infusions. *J Vet Emerg Crit Care (San Antonio)*. 2016;26:509-515.
19. Schaden E, Wetzel L, Kozek-Langenecker S, Thaler U, Scharbert G. Effect of the carrier solution for hydroxyethyl starch on platelet aggregation and clot formation. *Br J Anaesth*. 2012;109:572-577.
20. Arellano R, Gan BS, Salpeter MJ, et al. A triple-blinded randomized trial comparing the hemostatic effects of large-dose 10% hydroxyethyl starch 264/0.45 versus 5% albumin during major reconstructive surgery. *Anesth Analg*. 2005;100:1846-1853.
21. Kasper SM, Meinert P, Kampe S, et al. Large-dose hydroxyethyl starch 130/0.4 does not increase blood loss and transfusion requirements in coronary artery bypass surgery compared with hydroxyethyl starch 200/0.5 at recommended doses. *Anesthesiology*. 2003;99:42-47.
22. Neff TA, Doelberg M, Jungheinrich C, et al. Repetitive large-dose infusion of the novel hydroxyethyl starch 130/0.4 in patients with severe head injury. *Anesth Analg*. 2003;96:1453-1459.
23. Entholzner EK, Mielke LL, Calatzis AN, Feyh J, Hipp R, Hargasser SR. Coagulation effects of a recently developed hydroxyethyl starch (HES 130/0.4) compared to hydroxyethyl starches with higher molecular weight. *Acta Anaesthesiol Scand*. 2000;44:1116-1121.
24. Ferreira RR, Gopegui RR, Araujo MM, de Matos AJ. Effects of repeated blood donations on iron status and hematologic variables of canine blood donors. *J Am Vet Med Assoc*. 2014;244:1298-1303.

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