

In vitro effect of hydroxyethyl starch on coagulation in dogs as assessed by dynamic viscoelastic coagulometry

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OBJECTIVE

To evaluate the effect of 6% hydroxyethyl starch (HES) 670/0.75 and 6% HES 130/0.4 dilution of canine whole blood on coagulation using dynamic viscoelastic coagulometry (DVC).

ANIMALS

56 healthy adult dogs.

PROCEDURES

2 blood samples were obtained from each dog and randomized to 1 of 7 groups—undiluted or 2 dilutions (1:3 or 1:10) of 3 different fluids: saline (0.9% NaCl) solution, 6% HES 670/0.75, or 6% HES 130/0.4. Dilutions were calculated to simulate approximately a 10- or 30-mL/kg body weight IV bolus of each fluid. DVC was performed on each sample. Coagulation parameters compared between groups included clot rate (CR), platelet function (PF), and activated clotting time.

RESULTS

Dilution with saline solution did not significantly affect coagulation, while dilution with HES 670/0.75 and HES 130/0.4 caused a dose-dependent significant decrease in CR (1:3 HES 670/0.75, $P = 0.007$; 1:10 HES 670/0.75, $P = 0.002$; 1:3 HES 130/0.4, $P < 0.0001$; and 1:10 HES 130/0.4, $P = 0.0003$) and PF (1:3 HES 670/0.75, $P < 0.0001$; 1:10 HES 670/0.75, $P < 0.0001$; 1:3 HES 130/0.4, $P < 0.0001$; and 1:10 HES 130/0.4, $P = 0.0015$).

CLINICAL RELEVANCE

Dilution of canine blood with HES 670/0.75 and HES 130/0.4, at clinically relevant doses (10 and 30 mL/kg), led to significant hypocoagulability beyond dilutional effect. This was, in part, due to impaired PF, which was significantly greater with HES 670/0.75. Further research using DVC to assess the effects of HES on coagulation in dogs, ideally with clinical conditions warranting HES administration, is needed.

Intravenous crystalloid and synthetic colloid fluid therapies are commonly used in veterinary medicine for volume resuscitation, oncotic support, and rehydration. Administration of IV fluid therapies, most notably synthetic colloids, might lead to unintended consequences such as alterations of coagulation.¹⁻¹¹ In general, synthetic colloids such as hydroxyethyl starches (HES) cause relative hypocoagulability, which is at least partly due to impairment of platelet function (PF).^{6,7,9,11,12} This has been demonstrated in vitro and in vivo in dogs after an IV bolus or constant rate infusion.^{4-7,9,11} These coagulation changes are seen in healthy dogs with induced systemic inflammation and induced hemorrhagic shock and those undergoing general anesthesia for orthopedic procedures or imaging studies.^{5,6,8,9} Few studies have investigated the effects of synthetic

colloid administration on coagulation in dogs with naturally occurring systemic disease, although one study¹⁰ failed to detect a significant effect on coagulation in dogs with hypoalbuminemia. The clinical significance of the changes to coagulation remains unknown, as evidence of spontaneous hemorrhage or bleeding tendencies following HES administration, has not been reported.⁸⁻¹⁰

Hydroxyethyl starches are classified based on concentration, average molecular weight, degree of substitution (the average number of hydroxyethyl groups per glucose unit), and C2:C6 ratio (relative substitution at the position C2 compared with C6).^{13,14} Hydroxyethyl starches most commonly used in small animal veterinary medicine range from 675 kDa with 0.75 degree of substitution to 130 kDa with 0.4 substitution rates. In dogs receiving an

IV bolus, HES effects on coagulation can last > 24 hours with HES 600 to 675/0.75 and generally last less than 24 hours with HES 130/0.4.⁵⁻⁷

Dynamic whole blood viscoelastic tests of coagulation have been used in dogs to assess coagulation in health and disease. Commercially available devices include rotational thromboelastometry, thromboelastography, and dynamic viscoelastic coagulometry (DVC; Sonoclot coagulation and platelet function analyzer; Sienco Inc). All of these devices provide information regarding clot dynamics—rate of clot formation, strength of the clot, and clot lysis. To date, all published dynamic whole blood viscoelastic coagulation studies^{1-6,10,11,15} on the effects of synthetic colloids in dogs have used thromboelastography or thromboelastometry. Additional studies^{6,7,9,11,12} have specifically assessed PF using thromboelastography or rotational thromboelastometry.

Dynamic viscoelastic coagulometry uses a disposable cuvette and hollow pin that oscillates vertically within the blood sample, detecting changes to viscoelasticity as the clot forms. It provides a signature tracing, which is a graphical representation of clot formation over time, and coagulation parameters including clot rate (CR), PF, and activated clotting time (ACT). Clot rate represents the initial fibrin polymerization and clot development and is related to fibrinogen concentration, quality of thrombin, and PF.^{16,17} Platelet function is provided as a value between 0 and 5, which is determined based on the timing and quality of clot retraction.^{16,17} In humans, PF significantly correlates to platelet number and function as assessed by aggregometry.¹⁸⁻²⁰ In healthy dogs, PF significantly correlates to platelet count, but there are no published data comparing it to other tests of PF.²¹ Activated clotting time is the time to the beginning of fibrin formation and is related to soluble coagulation factors and endogenous anticoagulants.¹⁷

It is unknown if DVC can be used to assess the effects of HES on coagulation in dogs. This study was designed to test the hypothesis that the addition of HES 670/0.75 and HES 130/0.4 to canine whole blood would lead to a dose-dependent relative hypocoagulability that could be demonstrated with DVC.

Materials and Methods

Animals

This study was approved by the Institutional Animal Care and Use Committee at Oklahoma State University. Healthy dogs owned by students, faculty, and staff of the Veterinary Medical Hospital at Oklahoma State University were recruited for the study. Signed and informed pet-owner consent was obtained before study inclusion. Inclusion criterion included being considered healthy based on provided medical history, physical examination, and evaluation of a CBC, serum biochemistry analysis, prothrombin time (PT), and partial thromboplastin time (PTT). Exclusion criterion included administration of a medication known to affect coagulation, including, but not

limited to, nonsteroidal anti-inflammatories, corticosteroids, aspirin, clopidogrel, warfarin, or heparin during the month prior to enrollment.

Blood collection

Blood was collected from each dog via a traumatic stick jugular venipuncture using a 20-gauge, 1-inch needle and 6-mL syringe. Two 4-mL blood samples were obtained with at least 1 h between collections. The second sample was drawn from the contralateral jugular vein. A CBC (AU680 Clinical Chemistry Analyzer; Beckman Coulter; Antech Diagnostics), serum biochemistry analysis (AU680 Clinical Chemistry Analyzer), PT (KC4 Delta; Tcoag; Antech Diagnostics), and PTT (KC4 Delta) were performed on the first sample obtained. Platelet estimates were automated; if low, a manual platelet count was performed by a laboratory technician and reviewed by a clinical pathologist. Each sample was placed into 2 commercially available blood tubes (BD Vacutainer citrate tubes) containing 3.8% trisodium citrate (1 part citrate: 9 parts blood) and stored upright, without agitation, at room temperature until DVC analysis.

Study design

This study was a partially balanced, incomplete random block design with each blood sample being randomly assigned to 1 of 7 groups: (1) no dilution; (2) 1:3 dilution with saline (0.9% NaCl) solution (9% sodium chloride injection, Baxter Healthcare Corp); (3) 1:10 dilution with saline solution; (4) 1:3 dilution with 6% HES 670/0.75 (Hespan; Hospira Inc) in saline solution; (5) 1:10 dilution with 6% HES 670/0.75 in saline solution; (6) 1:3 dilution with 6% HES 130/0.4 (Vetstarch; Zoetis) in saline solution; and (7) 1:10 dilution with 6% HES 130/0.4 in saline solution. The dilutions were equivalent to a 10- or 30-mL/kg IV bolus of each fluid, which was similar to dilutions used in previous *in vitro* studies^{1-3,11,12,22} in dogs.

Dynamic viscoelastic coagulometry

A single dual-chamber dynamic viscoelastic coagulometer (Sonoclot coagulation and platelet function analyzer; Sienco Inc) was used for all DVC analyses performed by one operator. Routine maintenance and quality control procedures included daily calibration with a reference viscosity standard, monthly evaluation of control plasma samples, and visual inspections of signature tracings. Glass bead-activated cuvettes (gbACT+; Sienco Inc) were used for all assays. For control samples, 330 μ L of citrated whole blood was added to 30 μ L of 0.2 M CaCl_2 (Fisher Chemical) in a cuvette warmed to 37 °C. For 1:3 dilution samples, 248 μ L of citrated whole blood and 90 μ L of diluent (saline solution, HES 670/0.75, or HES 130/0.4) were added to 22 μ L of 0.2 M CaCl_2 in a cuvette warmed to 37 °C. For 1:10 dilution samples, 300 μ L of citrated whole blood and 33 μ L of diluent (saline solution, HES 670/0.75, or HES 130/0.4) were added to 27 μ L of 0.2 M CaCl_2 in a cuvette warmed to 37 °C. All DVC analyses lasted for 30 minutes or until the CR, PF, and ACT were reported.

Table 1—Results of dynamic viscoelastic coagulometry for whole blood samples from 56 healthy dogs after in vitro dilution of samples with synthetic colloids or saline (0.9% NaCl) solution.

Parameter/Fluid	Dilution	Mean	SE
CR (U/min)			
Control	NA	19.7 ^a	1.2
Saline	1:10	20.0 ^a	1.3
Saline	1:3	21.7 ^a	1.8
HES670	1:10	14.4 ^{b,c}	1.3
HES670	1:3	15.1 ^b	0.9
HES130	1:10	13.6 ^{b,c}	1.1
HES130	1:3	10.5 ^c	0.8
PF (unitless)			
Control	NA	3.3 ^a	0.1
Saline	1:10	3.4 ^a	0.1
Saline	1:3	3.3 ^a	0.1
HES670	1:10	2.4 ^c	0.1
HES670	1:3	1.4 ^e	0.1
HES130	1:10	2.8 ^b	0.1
HES130	1:3	1.9 ^d	0.1
ACT (s)			
Control	NA	143.5 ^a	5.1
Saline	1:10	121.8 ^{a,b}	7.2
Saline	1:3	111.6 ^b	5.9
HES670	1:10	125.2 ^{a,b}	7.6
HES670	1:3	128.9 ^{a,b}	11.4
HES130	1:10	131.9 ^{a,b}	6.4
HES130	1:3	118.8 ^b	7.0

ACT = Activated clotting time. CR = Clot rate. HES130 = Hydroxyethyl starch 130/0.4. HES670 = Hydroxyethyl starch 670/0.75. NA = Not applicable. PF = Platelet function.

^{a-e}Mean values with different superscript letters denote a significant ($P < 0.05$) difference.

Statistical analysis

Statistical analysis was performed with commercial software (SAS 9.4; SAS Institute, Cary, NC). Descriptive statistics were presented as median (range) or mean (SE). Mean DVC coagulation parameters (ACT, CR, and PF) were compared between groups using ANOVA with protected pairwise least significant difference. Statistical significance was set at $P < 0.05$.

Results

Animals and routine laboratory work

Fifty-six dogs with a median age of 4 years (range: 1 to 8 years) and median body weight of 20.4 kg (range, 4.8 to 63.8 kg) were included in the study. There were 32 females (5 sexually intact and 27 spayed) and 24 males (1 sexually intact and 23 castrated). Mixed breed dogs ($n = 18$) and 23 different purebred dogs were represented (38).

All venipuncture was performed appropriately for adequate processing of samples and adequate reporting. All assays were also performed without encountering any concerns. Prothrombin time, PTT, and Hct were within reference intervals for all dogs. Platelet counts were within reference intervals or estimated to be adequate with platelet clumping in all dogs. Mild abnormalities on CBC or serum biochemistry analysis were found in 5 dogs, including hyperglobulinemia in 2 dogs (4.3 and 4.3 g/dL; reference range, 1.6 to 3.6 g/dL), increased serum alanine aminotransferase activity in 1 dog (164 U/L; reference range, 12 to 118 U/L), and eosinophilia in 2 dogs (1,204 and 1,515 cells/ μ L; reference range, 0 to 1,200 cells/ μ L). These abnormalities were considered clinically insignificant, and the dogs were included in the study.

Dynamic viscoelastic coagulometry

Coagulation results were tabulated (Table 1). In summary, both HES 670/0.75 and HES 130/0.4 at 1:10 and 1:3 dilutions resulted in a significant decrease in CR when compared with no dilution or dilution with an equal volume of saline solution (Figure 1). Saline solution at either dilution did not significantly affect CR when compared with no dilution. Both HES 670/0.75 and HES 130/0.4 at both dilutions caused a significant decrease in PF as compared with no dilution and dilution with equal volumes of saline solution (Figure 2). Saline at

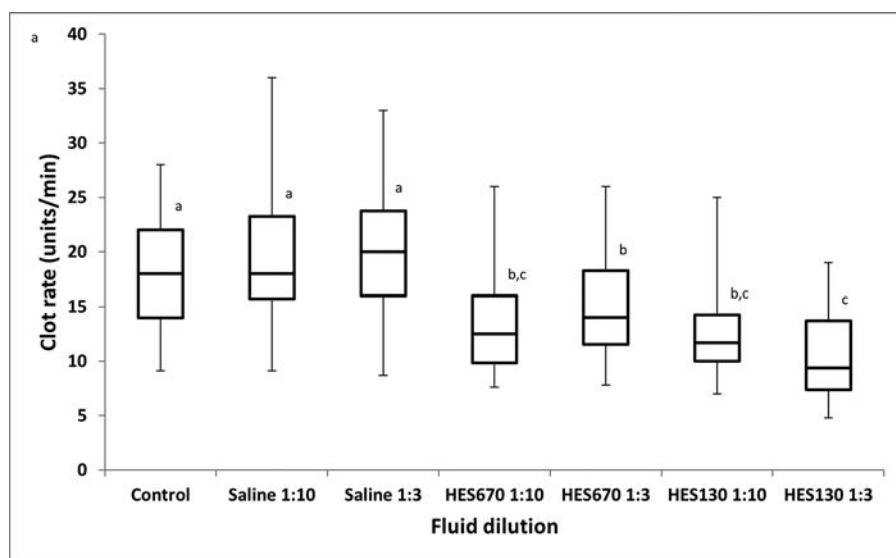


Figure 1—Box-and-whisker plots of clot rate as measured by dynamic viscoelastic coagulometry in whole blood samples from 56 healthy dogs diluted with synthetic colloids or saline (0.9% NaCl) solution. The central horizontal line within each box represents the median, the box represents the interquartile (25th to 75th percentile) range, and the whiskers represent the full range of data (0 to 100th percentile). HES130 = Hydroxyethyl starch 130/0.4. HES670 = Hydroxyethyl starch 670/0.75. ^{a-c}Variables with the same letters are not significantly ($P < 0.05$) different.

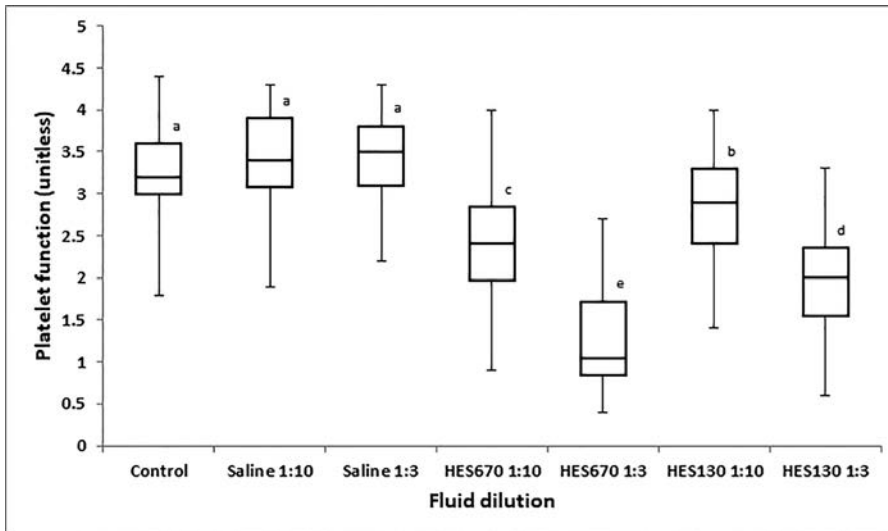


Figure 2—Box-and-whisker plots of platelet function as measured by dynamic viscoelastic coagulometry in the whole blood samples of Figure 1. ^{a-e}Variables with the same letters are not significantly ($P < 0.05$) different.

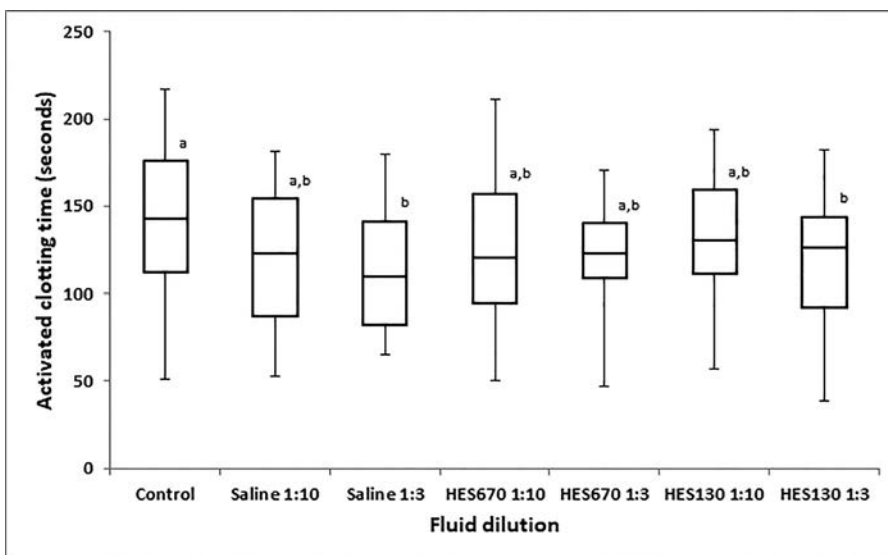


Figure 3—Box-and-whisker plots of activated clotting time as measured by dynamic viscoelastic coagulometry in the whole blood samples of Figure 1. See Figure 1 for remainder of key.

either dilution did not significantly affect PF when compared with no dilution. Only saline solution at the 1:3 dilution and HES 130/0.4 at the 1:3 dilution caused a significant decrease in ACT compared with no dilution (**Figure 3**). These were not significantly different from ACT with other dilutions of saline, HES 670/.75, or HES 130/0.4.

Discussion

To the authors' knowledge, this was the first study to investigate the effects of HES on canine whole blood coagulation using DVC. In this study, dilution of canine whole blood with either HES

670/0.75 or HES 130/0.4 led to relative hypocoagulability that was partly due to significantly impaired PF. The fact that an equal volume of saline solution did not lead to similar findings suggests that the underlying cause of hypocoagulability goes beyond simple dilutional effects, platelet consumption, or other preanalytical factors, such as venipuncture technique, incubation time, and incubation temperature.

In this study, PF was significantly decreased with both dilutions of both HES solutions. There was a dose-dependent effect, as the higher dilutions led to significantly lower PF. The effects on PF were immediate, as the HES and whole blood were mixed at the initiation of the DVC analysis. This corroborates previous findings in dogs and humans where relative impairment of PF has been described with HES administration or dilution.^{1,6,7,9,11,12,23} Conflicting results have been reported concerning the effects of HES 130/0.4 on PF in dogs.^{3,4,22,24} In those studies, the coagulopathies caused by HES 130/0.4 were no more than those caused by dilutional effects (saline or lactated Ringer solution). Some of those studies included samples from a small number of dogs (6 to 27 dogs) and might have been underpowered to detect smaller effects. Other possible reasons for conflicting results include variable doses or

dilutions of HES, different coagulation devices or PF analyzers, different activators, and other preanalytical and analytical variabilities.

This study demonstrated significantly greater impairment of PF with HES 670/0.75 as compared with equal volumes of HES 130/0.4. This contradicts a previous *in vitro* study¹¹ that failed to show a significant difference in PF between HES 670/0.75 and HES 130/0.4 in canine blood. Overall, there is reason to believe that HES solutions with higher molecular weights and degrees of substitution do lead to relatively more impairment of PF, as findings of impaired PF have been more consistent with HES 670/0.75, compared with HES 130/0.4.^{1,3,4,6-9,12,22} It has been

shown that higher molecular weight, degree of substitution, and C2:C6 ratio lead to slower metabolism and thus a longer duration of effect.^{13,25,26} In addition, one canine study²² found significantly greater impairment of PF with HES 200/0.5, when compared with an equal volume of HES 130/0.4. Findings of relatively greater impairment of PF with a higher molecular weight and degree of substitution HES solution in the present study are consistent with previously reported data in humans.^{27,28}

Proposed reasons that HES might cause impairment of PF include inhibition or decreased expression of glycoprotein (GP) IIb/IIIa, also known as integrin $\alpha\text{IIb}\beta_3$, and decreased expression of cell surface serine phospholipids, among others.^{29,30} Glycoprotein IIb/IIIa plays a significant role in platelet adhesion and aggregation. Once a platelet is activated, GP IIb/IIIa undergoes a conformational change to allow for an increased affinity of the receptor for binding of fibrinogen. With fibrinogen binding, adjacent platelets can undergo a facilitated interaction and GP IIb/IIIa undergoes further conformational change to allow for an acceleration of platelet aggregation.³¹ Dynamic viscoelastic coagulometry is a sensitive indicator of impaired PF secondary to GP IIb/IIIa inhibition, caused by tirofiban.¹⁹ Tirofiban also affects closure time, as measured by the Platelet Function Analyzer-100, a device commonly used in the published literature of the effects of HES in dogs.¹⁹ The findings of the present study suggested that DVC is a sensitive indicator of impaired PF due to HES in dogs. As such, advantages of DVC might include a single test that can provide a global view of whole blood coagulation along with easy-to-obtain information regarding the contribution of PF. Further research, including the *in vivo* effects of colloids on whole blood coagulation as assessed by DVC, is needed.

In addition to PF, CR was significantly prolonged with both dilutions of both HES solutions in the present study. This effect was not seen with equal volumes of saline solution. The significantly lower CR was likely, at least partly, due to the noted impaired PF. Different from PF, a dose-dependent effect was not seen with CR, which might suggest that a factor other than PF is also affecting CR. Clot rate is also affected by thrombin and fibrinogen concentrations. *In vitro* HES 130/0.4 has been shown to decrease factor VIII activity and serine phospholipid expression, thus decreasing thrombin generation.²⁹ Significant effects of thrombin or fibrinogen concentrations are not supported by relative lack of significant changes seen to ACT. As a result of design, in this study, investigation of changes to specific coagulation factor activity, phospholipid expression, thrombin generation, fibrinogen concentration, or other potential variables was not possible.

Activated clotting time is not significantly affected by platelet count or PF. In the present study, the changes to ACT were small and inconsistent between fluid types and dilutions. In general, HES dilution did not significantly affect ACT as compared with samples with no dilution or dilution with saline solution. This is consistent with other findings in this

study that suggest that impaired PF, and not simple dilutional effects, explain the relative hypocoagulability demonstrated with HES. As evaluated by DVC, collectively these findings suggest that HES has only relatively small effects on soluble coagulation factors and endogenous anticoagulants in canine blood.

Our study had multiple limitations, some of which are inherent to *in vitro* studies. These include the exclusion of endothelial cell, blood flow dynamics, HES pharmacokinetics, and many other complex physiological factors that are not recreated with DVC. A second limitation included the use of healthy dogs. In clinical practice, HES is expected to be commonly used in unhealthy dogs requiring volume expansion or oncotic support. Using healthy dogs excludes many possible pathologic factors that might affect coagulation, such as protein-losing disease, systemic inflammation, cancer, and immune-mediated disease, among others. A third limitation was that our study was designed to investigate a fluid bolus scenario and HES is also administered over longer periods of time clinically. Immediate platelet inhibition has been demonstrated in humans after a bolus of HES, suggesting that even short-term exposure to HES *in vitro* could mimic those *in vivo*. It is unknown how these effects would be altered by longer duration lower concentration exposure, as could be seen with a constant rate infusion of HES.³² A final limitation included the fact that an additional PF analyzer was not used. Although there is evidence that DVC demonstrates PF in humans,¹⁸⁻²⁰ it has not been validated for PF testing in dogs.

In conclusion, dilution of canine blood with HES 670/0.75 and HES 130/0.4 at clinically relevant doses (10 and 30 mL/kg) led to significant hypocoagulability beyond simple dilutional effects as evaluated by DVC. This was at least partly due to impaired PF, which was significantly greater with HES 670/0.75 as compared with HES 130/0.4. Further research using DVC to assess the effects of HES on coagulation in dogs, ideally with clinical conditions warranting HES administration, is needed.

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