**Assessment of hemostasis in dogs with shock after administration of hydroxyethyl starch (130/0.4) or hypertonic saline (7.5%)**

## Abstract

**Objective** – Compare the impact on hemostasis of intravenous bolus of hydroxyethyl starch 130/0.4 (HES) or hypertonic saline 7.5% (HS) in dogs resuscitated for gastric dilation volvulus (GDV).

**Design –** Open label parallel group randomized clinical trial

**Animals** – 23 client-owned dogs.

**Interventions** – Dogs affected by GDV and shock, were randomly assigned to receive HES at 10 ml/Kg or HS at 4 ml/Kg per 15 minutes. Blood samples were collected for blood gas analysis, PCV, total protein, albumin, standard coagulation profile and thromboelastometry (ROTEM) at baseline (T0), and at the end of bolus (T1). To assess the differences between the two groups at T1, t-Student test or Wilcoxon rank-sum test were used. To evaluate the differences between T0 and T1, ANOVA for paired data or the Wilcoxon matched-pairs signed-ranks test were used. A value of $P<0.05$ was considered for significance.

**Measurement and Main Results** – Hemostasis was evaluated by means of prothrombin time, activated partial thromboplastin time, fibrinogen and ROTEM.

The study included 13 dogs in HES group and 10 dogs in HS group. Significant differences between groups at T1: increase of clotting time ($P=0.018$) and decrease in fibrinogen level ($P=0.021$) in the HS-treated group.

Significant differences between T0 and T1: increase of clot formation time ($P = 0.046$), decrease in maximum clot firmness ($P = 0.002$) in ex-TEM profile, and decrease of maximum clot firmness ($P=0.0117$) in fib-TEM profile, for HES group; increase of clotting time ($P=0.048$) and clot formation time (0.0019), decrease of maximum clot firmness ($P=0.031$) and α angle

(P=0.036) in ex-TEM profile, decrease in α angle (P=0.036) in in-TEM profile, and decrease in maximum clot firmness (P=0.017) in fib-TEM profile, for HS group.

**Conclusion –** In dogs affected by GDV, the doses of HES or HS administered have caused few differences in hemostatic analyses, indicating a similar tendency to hypocoagulability.

## ABBREVIATIONS

ALB: albumin

ANOVA: analysis of variance

aPTT: activated partial thromboplastin time

APPLE fast score: acute patient physiologic and laboratory evaluation fast scoring system

CT: clotting time

CFT: clot formation time

Ex-TEM: extrinsic thromboelastometry pathway

Fib-TEM: functional fibrinogen

GDV: gastric dilation and volvulus

Hct: hematocrit

HES: hydroxyethyl starch

HS: hypertonic saline

In-TEM: intrinsic thromboelastometry pathway

DIC: disseminated intravascular coagulation

MCF: maximum clot firmness

$MCE_{PLT:}$ platelet contribution to maximum clot elasticity

PCV: microhematocrit

PT: prothrombin time

ROTEM: rotational thromboelastometry

TEG: thromboelastography

T0: blood sample collected after application of catheter in cephalic vein

T1: blood sample collected after the bolus

TP: total protein

**Key words:** coagulation, thromboelastometry, hydroxyethyl starch, hypertonic saline

## Introduction

Intravenous (IV) fluid therapy for resuscitation from cardiovascular shock differs by type of fluid, dosage, side effects, and indications. The two major categories are represented by crystalloid and artificial colloids solutions.[1]

Hydroxyethyl starches (HES) are artificial colloid frequently used in veterinary medicine for intravascular volume expansion. In human medicine, adverse effects, as coagulopathies, kidney injury, and tissue storage, are reported after HES administration, which seem to be related to mean molecular weight, molar substitution, and C2/C6 ratio.[2-4] Hemostatic alterations mainly result from hemodilution, but a direct effect of HES macromolecules, such as platelet dysfunction with decreased expression of integrin αIIbβ3, a reduction in clotting factor activities (e.g., factor VIII and von Willebrand factor), a decrease in fibrinogen polymerization, and impaired

fibrinolysis is also involved.[5] Both *in vitro* and *in vivo* veterinary studies have investigated hemostatic alterations in dogs after blood dilution or intravenous administration of HES respectively, demonstrating a HES dose and HES type dependent decrease in platelet aggregation and hypocoagulability; none to date have reported clinical bleeding.[6-16] Despite this, comparing those studies is challenging, as HES preparations with different molecular weight, degree of substitution, and dosages have been used.

Hypertonic saline (HS), a type of crystalloid solution with high osmolality, is mainly indicated for *small volume* fluid resuscitation in patients with head trauma or hypovolemic shock.[1,17] Hypertonic saline administration might be associated with advantages as reducing endothelial swelling, improving cardiac output, and modulation of inflammation, but its rapid administration (1 ml/kg/min) may cause bradycardia, hypotension and vomiting.[18-21] In humans, impairment of coagulation has also been reported and three recent veterinary studies, conducted in dogs, have shown a decrease in platelet function and hypocoagulability after HS dilution.[13,22-25]

Tight connection between inflammation, shock and coagulation can cause changes in hemostasis of critically ill patients causing bleeding or thrombosis, which complicate management and can affect prognosis of these kinds of animals. Also the amount and type of infusion solutions administered can have an impact on coagulation, and it is important to known the magnitude and the clinical relevance of this interaction, to take it into account during the resuscitation to anticipate possible complications and make a monitoring plan.[26,27]

In dogs with GDV, hemostatic abnormalities, as disseminated intravascular coagulation (DIC), have been reported in prospective and retrospective studies that used a standard coagulation

profile.[a,28-33] The relative superiority of HES *versus* HS, as a resuscitation fluid for minimizing induced hypocoagulability in dogs with GDV, is unknown.

The aim of this study was to compare the impact on hemostasis of fluid resuscitation with an intravenous bolus of either HES (130/0.4) or HS (7.5%) in dogs with GDV, by means of rotational thromboelastometry.

Our hypothesis was that HES solution will impair coagulation more than HS solution, leading to a hypocoagulable state which could further complicate the management of dogs hemodynamically unstable.

## Materials and Methods

### Animals

The study protocol was approved by the Bioethical Committee of University (protocol number 47077 and DL 26/2014, Project 581). This prospective, randomized, multicenter investigation involved client-owned dogs. The owner gave their written, informed consent for participation.

All dogs enrolled were patients admitted by Veterinary Teaching Hospital for suspected GDV syndrome based on clinical signs. Inclusion criteria were: diagnosis of GDV based on history, clinical signs, abdominal radiographs, and surgical exploration, and evidence of cardiovascular shock [detected by heart rate >130 bpm, poor pulse quality, capillary refill time > 2 s or < 1 s and venous lactate >2 mmol/L (18 mg/dl)]. Exclusion criteria were: administration of non-steroidal anti-inflammatory drugs, corticosteroid, and artificial colloid or blood products in the 4 weeks prior to enrolment in the study, and/or history of cardiac, pulmonary, renal or liver failure.

At presentation, clinical data were collected, including recent history and a complete physical examination. Whole blood samples were collected to perform laboratory analyses. For each dog the acute patient physiologic and laboratory evaluation fast score (APPLE fast score) was calculated on admission, to define illness severity as previously described.[34]

After application of a catheter in each cephalic vein, blood samples (T0) were collected from jugular vein for CBC [b], biochemical evaluation [c], venous blood gas analysis (including electrolytes) [d], packed cell volume, total solids, standard coagulation profile [prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen] [e] and rotational thromboelastometry (ROTEM) [f]. Intravenous fluid therapy with crystalloids solution (15 ml/Kg per 15 minutes of Ringer lactate solution) was then administered together with methadone 0.2 mg/Kg IV, and thoracic and abdominal radiographs were obtained.

After confirmation of GDV, the dogs were randomly assigned [g] to receive either HES 130/0.4 [h] at 10 ml/Kg or HS 7.5% [i] at 4 ml/Kg per 15 minutes. If necessary, percutaneous decompression of the stomach was performed during the bolus. On completion of the bolus, whole blood was collected again (T1) for blood gas analysis, packed cell volume, total solids, albumin (ALB), standard coagulation profile and ROTEM analysis.

Respiratory rate, heart rate (combined with a constant electrocardiogram monitoring), capillary refill time, metatarsal pulse quality, systolic blood pressure, and rectal temperature were evaluated during all treatment phases.

After the end of the study, isotonic crystalloids solution administration was continued at the discretion of the attending physician until the dog was stable enough to undergo anesthesia and surgery.

## Assessment of Hemostasis

Whole blood samples were collected by jugular venipuncture (20-gauge needle) and placed into two tubes containing 3.2% trisodium citrate (1 part citrate: 9 parts blood). Samples obtained after repeated venipuncture attempts, needle repositioning or interruption of blood flow into the tube, were discarded and blood draws were made from the contralateral jugular vein.

Secondary hemostasis was evaluated by means of standard plasma-based assays (PT, aPTT and fibrinogen). Thromboelastometric analyses were performed according to PROVETS guidelines and the analyses were running for 30 minutes.[35,36] For each sample, in-TEM, ex-TEM and fib-TEM profiles were performed to evaluate the intrinsic pathway (activation by ellagic acid), the extrinsic pathway (tissue factor activation), and functional fibrinogen (platelets inactivated with cytochalasin D), respectively. The following parameters were assessed for each profile: clotting time ([CT], s); clot formation time ([CFT], s); maximum clot firmness ([MCF], mm); α angle (α, °); profiles are represented as reaction curves (Fig. 1). CT represents the first fibrin clot formed from activation of the test until clot amplitude of 2 mm; this parameter is affected by the concentration of plasma coagulation factors and coagulation inhibitors (e.g., antithrombin or anticoagulant drugs).[37,38] CFT expresses the velocity of clot formation and is influenced by platelet count, function and by fibrinogen activity. MCF, the maximum firmness the clot reaches, is determined by both platelet count, function and fibrin formation in the presence of factor XIII. [37,38] The α angle corresponds to the slope of the tangent on the elasticity curve; it describes the kinetics of clot formation and is affected predominantly by platelet count, function and fibrinogen.[37,38] The reference ranges for ROTEM parameters were previously established at our institution in 45 healthy dogs.[9]

153 An additional calculated parameter is $MCE_{PLT}$ (platelet contribution to maximum clot
154 elasticity), which evaluates platelet contribution to clot elasticity, and is obtained as follows:
155 $MCE_{PLT}$= $MCE_{extem}$-$MCE_{fibtem}$ [MCE=(MCF*100)/(100-MCF) ].[39] After platelets have bound to
156 fibrin via the glycoprotein IIb/IIIa receptor, the clot contracts through the action of cytoplasmic
157 motility proteins inside platelets, such that serum is expelled; these clot contractile forces may
158 contribute to clot stiffness.

159 Hypercoagulable ROTEM tracing is characterized by decrease of CT or CFT and increase
160 MCF or α angle, whereas hypocoagulable tracing is distinguished by increase in CT or CFT and
161 decrease in MCF or α angle indicate.

162 **Study design and statistical analysis**

163 The study was designed as a parallel group completely randomized open label design: subjects
164 were randomly allocated to receive one of the two treatments. The eligibility criteria are stated in
165 the "Animals" paragraph. A priori power analysis was calculated to determine the sample size
166 required. The criteria were: statistical significance level at 5%; power at 80%; delta [difference
167 between the two groups mean value of one of the coagulative parameters as expressed in Wurlod
168 V. A. et al., (2015)] equal to 3 and standard deviation equal to 2.4.[15]

169 Data were entered in an ad hoc database, analyzed with Stata 15 [1] (Stata Statistical Software:
170 StataCorp LP, College Station, Texas USA), and tested for Normality by Shapiro-Wilk test. To
171 assess the equivalence of the two groups at T0 and, separately, to assess the differences in
172 treatment between the two groups at T1, t-Student test was performed when data resulted
173 Normally distributed, otherwise the Wilcoxon rank-sum test was used. To assess the differences
174 between T0 and T1, ANOVA for paired data was used when data were normally distributed;

otherwise the Wilcoxon matched-pairs signed-ranks test was used. A value of $P<0.05$ was considered for significance.

## Results

Twenty-six dogs were included in the study: 13 in the HES-treated group and 13 in the HS-treated group. Three patients in the HS group were excluded: 2 for technical reasons (ROTEM malfunction), and another one that died before the end of protocol. The HES-treated group was composed of 7 females (2 entire and 5 spayed) and 6 males (5 intact and 1 neutered), the median age was 10 years (min 1-max 13) and the median body weight was 35 kg (min 17-max 55); breeds included were: Bloodhound (n=1), Boxer (n=1), Chow chow (n=1), Hound dog (n=1), Italian Mastiff (n=1), Pyrenean Mountain Dog (n=1), Dobermann (n=2), Mixed breed (n=2) and German shepherd (n=3). The HS-treated group included 4 females (1 entire and 3 spayed) and 6 males (5 intact and 1 neutered), the median age was 10.5 years (min 2-max 14) and the median body weight was 37 kg (min 20-max 61); breeds comprised were: Bull Mastiff (n=1), Great Dane (n=1), Leonberger (n=1), Pit bull (n=1), German shepherd (n=2) and Mixed breed (n=4).

*Results at baseline (T0)*

Rotational thromboelastometry and laboratory results parameters of interest are presented in tables 1. At baseline (T0), the HES-treated group was characterized by: 1/13 was anemic (PCV < 37%), 2/13 were thrombocytopenic [platelets $<128x10^9$/L ($<128x10^3$cell/µL)], 7/13 had ALB level outside the lower reference range [ALB <0.3 g/L (<3 g/dl)], and 5/13 had lactate > 6 mmol/L (54 mg/dl). None of the dogs had PT or aPTT outside the upper reference range. Fibrinogen concentration was below the reference range [< 4.4 µmol/L (<150 mg/dl)] in 1/13 dogs and above [>13.2 µmol/L (<450 mg/dl)] in 1/13 dogs. (Table1) The median value of

APPLE fast score was 24 (min 18-max 41), 4/13 dogs had gastric necrosis, 3/13 underwent gastrectomy, and 1/13 was euthanized for economic reasons.

At T0, the HS-treated group was characterized by: no anemic dog (PCV < 37%), 1/10 was thrombocytopenic [(platelets <128x$10^9$cell/L (<128x$10^3$cell/µL)], 5/10 had an ALB level outside the lower reference range [ALB 0.3 g/L (< 3 g/dl)], and 5/10 had lactate > 6 mmol/L (54 mg/dl). None had PT or aPTT outside the upper reference range; the fibrinogen level was low in 1/10 [<4.4 µmol/L (<150 mg/dl)]. (Table1) The median value of APPLE fast score was 22.5 (min 10-max 40), 1/10 dogs had gastric necrosis, 1/10 underwent gastrectomy, and 4/10 were euthanized for economic reasons.

The two groups were similar at T0 with no statistically significant difference at baseline for any of the parameters.

At T0, most dogs of both groups had ROTEM tracings classified as normal, except 3 dogs in the HES-treated group. In these dogs, hypercoagulability was detected in 1/13 and hypocoagulability in 2/13 dogs. Both hypocoagulable dogs had normal PT, aPTT, and low platelet count, whereas one had a low fibrinogen concentration (Table 2).

*Comparison between results at T1*

Table 3 presents ROTEM values, standard coagulation profile, and laboratory analysis obtained at T1, and results of comparisons. A statistically significant difference was found between CT (p=0.018) in the in-TEM profiles of the two groups, with an increase in this parameter in the HS-treated group in comparison to HES- treated group.

After bolus (T1), a statistically significant decrease was found in fibrinogen level (P=0.021), PCV (P=0.002), pH (P=0.013), and a statistically significant increase was shown in chloride

(P=0.024) and sodium (P=0.006); whereas no difference was found between HES and HS-treated groups in $MCE_{PLT}$, PT, aPTT, TP and ALB (see Table 3).

*Comparison between T0 and T1*

Statistically significant differences between T0 and T1 in the HES-treated group were: increase in CFT (P = 0.046), decrease in MCF (P = 0.002) in the ex-TEM profile, and decrease in MCF (P=0.0117) in the fib-TEM profile. No difference was found between PT and aPTT concentration, whereas statistically significant decrease in fibrinogen level was observed (P=0.0005).

Statistically significant differences between T0 and T1 in the HS-treated group were: increased CT (P=0.048) and CFT (0.0019), and decreased MCF (P=0.031) and α angle (P=0.036) in the ex-TEM profile; decrease in α angle (P=0.036) in the in-TEM profile; decrease in MCF (P=0.017) in the fib-TEM profile, and decrease in $MCE_{PLT}$ (P=0.021). No difference was found in aPTT, whereas there was a statistically significant increase in PT (P=0.0039) and statistically significant decrease in fibrinogen concentration (P=0.027).

After HES bolus, a statistically significant decrease was found in PCV (P=0.003), TP (P=0.0005) and ALB (P=0.0002); whereas statistically significant increase was shown in chloride (P=0.0005).

After HS bolus, statistically significant decrease was found in PCV (P=0.0001), TP (P=0.0028), and ALB (P=0.0044); whereas statistically significant increase was shown in chloride (P=0.0003), sodium (P=0.0008).

*Hypocoagulable ROTEM of two dogs at T1*

Rotational thromboelastometry tracings of the two previously described hypocoagulable dogs at T0 in the HES group showed a hypocoagulable state after HES administration, with a further decrease in fibrinogen level in dog number 4 and an increase in PT and aPTT above the reference range in dog number 7 (Table 2). In these dogs, tendency to bleed was observed during or after surgery. Postsurgical abdominal bleeding was noted in dog 4 and the hemorrhage, hemodynamic instability, and coagulopathy were resolved with transfusion of fresh frozen plasma. Dog 7 experienced bleeding during surgery, followed by epistaxis and hemodynamic instability during recovery from anesthesia. The owners refused other treatment and opted for euthanasia.

## Discussion

The present study evaluated the effects on coagulation of two resuscitation fluids (HES and HS) administered as a bolus during the resuscitation phase in dogs affected by GDV.

Our results indicate that 10 ml/Kg of HES 130/0.4 and 4 ml/Kg of HS 7.5% administered over 15 minutes interferes with coagulation causing a tendency to hypocoagulability, that could be due to hemodilution and characteristics of fluids. However, comparison between the two groups at T1 has shown few differences on coagulation status, assessed with both ROTEM and standard coagulation profile. Although minimal, founded differences indicated that HS could interfere to a greater extent on coagulation. In particular, it was shown an increase in in-TEM CT in the HS-treated group compared to the HES-treated group. In-TEM CT represents the plasmatic phase of the intrinsic way, and considering the cell based model of coagulation, it has limited clinical relevance. In the standard coagulation profile, the only difference observed at T1 was a decrease in fibrinogen level in the HS-treated group compared to the HES-treated group, with no values below the 2.94 µmol/L (100 mg/dL).

A recent in vitro study has observed a dose dependent effect on hemostasis, following several dilutions of canine whole blood with isotonic crystalloid, hypertonic crystalloid and hydroxyethyl starch. Moreover, the results obtained have demonstrated that major alterations in hemostasis were observed with the HS, compare to the same dilution with HES. Despite the differences between the previous and our study, results obtained were similar.[40]

Two dogs in the HES-treated group identified as hypocoagulable by ROTEM analysis already at T0, have shown perioperative bleeding events. The hypocoagulable state has only partially related to direct HES effect, and other factors as dilution effect, aspects related to patient and illness characteristics may have acted together to impair coagulation.

Considering the single group of treatment, alterations observed between T0 and T1 in the HES-treated group, indicate a decrease in clot firmness and could be related to a decrease in fibrinogen concentration and platelet function. In ROTEM analysis, changes in CFT, α angle, and MCF parameters in particular can be influenced by some sample features such as platelet count, fibrinogen concentration, and Hct.[37] However, since a decrease in MCF in both ex-TEM and fib-TEM profiles and no change in $MCE_{PLT}$, could implied that fibrinogen impairment is the major determinant of these ROTEM changes. Indeed, the MCF of ex-TEM profile provides a measure of clot strength derived from both fibrin and platelets contribution, whereas in the MCF of fib-TEM profile, where addition of cytochalasin D prevents platelets activity, the clot strength derive from fibrinogen concentration and activity.

Similar results have been found in previous studies evaluating changes in hemostasis following HES 130/0.4 administration using different dosages and sample population of dogs. Results indicated that HES administration causes alterations to hemostasis, but changes reported

were not associated with clinical bleeding.[12,41,42]

In the HS-treated group, several ROTEM parameters were different between T0 and T1 with a tendency to hypocoagulability, and a decrease in $MCE_{PLT}$ was indicative of reduced platelet contribution to clot contraction/elasticity. Although, no difference was reported at T1 for the $MCE_{PLT}$ between the two groups, and further studies with increase in sample size or with different amount of solutions administered, could help to clarify this result.

Hyperosmolarity related to HS can reduces coagulation efficiency, interferes with platelet function and whole blood coagulation, and impairs clotting factors activity, fibrin formation and clot strength.[42-44] Recent in vitro and in vivo veterinary studies have demonstrated a dose-dependent HS effect on canine hemostasis.[13,24,25] In vitro studies have detected impairment of CFT and MCF in the ex-TEM profile of ROTEM analysis, and a recent in vivo study observing a decrease in CT in the fib-TEM profile and a decreased in platelet function, assessed using PFA-100.[13,24,25] Our results are consistent with previous studies, but some differences are present and could be explained by diverse amount of hypertonic crystalloid administered and dog populations selected. Indeed, our dogs were in cardiovascular shock and had hypovolemia, hypoperfusion, and acidosis, conditions that could affect hemostasis.[26,27]

At T1, fibrinogen level showed a significant decrease in the HS-treated group (within the reference interval). Interestingly, that result was not associated to change in fib-TEM profile between the two groups, indicating no changes in functional fibrinogen and neglectable clinical relevance. Standard coagulation profile assessment showed no significant changes in PT and aPTT between HS and HES-treated group.

Between T0 and T1, fibrinogen level had a significant decrease in both HS and HES-treated groups, whereas PT had a significant increase only in HS-treated group. Similar results on standard coagulation profile were observed by Seshia et al. (2018) after administration of 5 ml/Kg of HS over 15 min and 20 ml/Kg of HES over 30 minutes, in healthy dogs, making more likely that these changes were due to HS administration and not exclusive of our population.[45]

After the HS bolus administration, a significant increase in sodium and chloride, and a decrease in pH were showed in comparison with HES-treated group, indicating a worsening of acidosis and increase in osmolality, factors that might affected hemostasis in critically ill patients. Those results were expected and explained by the different characteristics of solutions.

Regarding the other laboratory parameters evaluated, a significant decrease in PCV, TS and ALB were noted in both groups from T0 to T1, indicating a potential hemodilution effect to both HES and HS administration, although the magnitude of hemodilution appeared greater in the HS-treated group, because PCV at T1 had a significant decrease in comparison with HES-treated group.

At baseline (T0), only ROTEM analysis was able to identified two dogs as hypocoagulable in the HES-treated group, whereas this alteration in hemostasis was not detected by PT and aPTT. After the bolus, the ROTEM values worsened and clinical bleeding developed in both dogs (during or after surgery), and the standard coagulation profile at T1 reflected hypocoagulability only in one dog. If ROTEM analysis cannot be perform, physicians should be pay special attention during resuscitation of GDV patient, because dogs could begin hypocoagulable after fluid administration and bleeding. Studies evaluating coagulation in dogs with GDV have reported multiple hemostatic abnormalities at hospital presentation, mainly indicative of

hypocoagulability, due to consumption of clotting factors and platelets caused by DIC.[31,33] One abstract published to date has described the use of a viscoelastic technique (TEG) in canine patients with GDV, reporting that dogs with baseline TEG values outside the reference range had higher mortality than dogs without abnormalities.[a]

The present study has several limitations. There was no control group treated only with isotonic crystalloids, that would allow to determine the amount of changes in hemostasis due to hemodilution versus a direct effect of HES or HS, and the trial were not blinded.

It would have been useful to determine the platelet count also at T1, to identify a decrease in platelet number that could influence with ROTEM parameters, although the $MCE_{PLT}$ assessment allowed for evaluation of platelet contribution.

Moreover, the hemostatic changes were evaluated after a bolus of HES or HS, and the effects on coagulation after their redistribution in the extravascular space or after administration of additional fluids are unknown.

In conclusion, results obtained in the present study indicate that the doses of HES or HS administered in dogs affected by GDV have determined similar impairment of hemostasis, but the few differences reported in the HS-treated group might suggest a greater effect of this solution.

In two dogs ROTEM analysis identified hypocoagulability condition at presentation, which worsened after the bolus and resulted in postoperative clinical bleeding; this status was not detected by the standard coagulation profile performed at T0, highlighting how the ROTEM is a more sensitive tool for the evaluation of coagulation.

Further studies are needed to better understand the dose-related effects of HES or HS administration on canine hemostasis and the clinical impact of these alterations.

**Acknowledgements**

The authors declare no conflicts of interest.

**Footnotes**

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b.ADVIA 120 Hematology, Siemens Healthcare Diagnostics, USA.

c. ILAB 300 plus, Clinical Chemistry System, Instrumentation Laboratories, Italy.

d. ABL 800 Flex; A. de Mori S.p.A., Italy.

e. Coagulometer StART, Diagnostica Stago, USA.

f. ROTEM, Tem International GmbH, Germany.

g. Microsoft Excel, Redmond, WA, USA.

h. Voluven, Fresenius Kabi Italia srl., Isola della Scala (VR), Italy.

i. NaCl 7.5%, B. Braun Melsungen, Melsungen, Germany.

l. Stata Statistical Software: Release 11. StataCorp LP, USA.

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496 **Table 1 –** Hemostasis assessment and laboratory parameters of interest at T0.

| **T0** | **HES GROUP** N=13 | **HS GROUP** N=10 | **P value** | **Institutional reference intervals** |
|---|---|---|---|---|
| ROTEM | | | | |
| **In-TEM** | | | | |
| CT (s) | 162 (127-365) | 170 (134-220) | 0.55 | 126-363 s |
| CFT (s) | 115 (40-368) | 88 (58-160) | 0.16 | 47-224 s |
| MCF (mm) | 58 (41-73) | 62 (50-72) | 0.62 | 50-75 mm |
| α angle (°) | 68 (41-82) | 74 (62-79) | 0.26 | 55-81 ° |
| **Ex-TEM** | | | | |
| CT (s) | 47 (30-169) | 40 (30-70) | 0.42 | 29-92 s |
| CFT (s) | 102 (44-365) | 85 (56-152) | 0.26 | 54-275 s |
| MCF (mm) | 62 (39-89) | 65 (54-81) | 0.66 | 36-73 mm |
| α angle (°) | 73 (33-83) | 75 (60-82) | 0.64 | 47-79 ° |
| **Fib-TEM** | | | | |
| CT (s) | 51 (28-59) | 39 (32-73) | 0.84 | 14-102 s |
| MCF (mm) | 12 (5-33) | 14 (10-24) | 0.15 | 6-26 mm |
| **$MCE_{PLT}$** | 156 (59-760) | 154 (100-409) | 0.73 | 50-235 |
| Standard coagulation | | | | |
| **aPTT (s)** | 12.4 (12-14.2) | 11.3 (9-15.2) | 0.47 | 12-16 s |
| **PT (s)** | 7.8 | 6.9 | 0.66 | 8-10 s |

| | | | | |
|---|---|---|---|---|
| | (6.1-9.5) | (6.3-9) | | |
| **Fibrinogen (µmol/L)** | 7.06 (3.8-11.8) [240 mg/dL (130-400)] | 5.6 (1.5-8.2) [190 mg/dL (50-280)] | 0.06 | 4.4-13.2 (µmol/L) [150-450 mg/dL] |
| Laboratory parameters | | | | |
| **PCV (%)** | 50 (30-55) | 43.5 (39-51) | 0.33 | 37.5-58.3 % |
| **Platelet count (x10$^9$cell/L)** | 168 (88-624) [168x10$^3$cell/µL (88-624)] | 239.5 (104-456) [239.5 x10$^3$cell/µL (104-456)] | 0.15 | 128-543 x10$^9$cell/L (128-543 x10$^3$cell/µL) |
| **Total Solid (g/L)** | 0.65 (0.58-0.92) [6.5 g/dl (5.8-9.2)] | 0.74 (0.52-0.89) [7.4 g/dl (5.2-8.9)] | 0.85 | 0.55-0.72 g/L (5.5-7.2 g/dl) |
| **Albumin (g/L)** | 0.29 (0.24-0.39) [2.9 g/dl (2.4-3.9)] | 0.3 (0.19-0.34) [3 g/dl (1.9-3.4)] | 0.24 | 0.3-0.39 g/L (3-3.9 g/dl) |
| **Chloride (mmol/L)** | 114 (82-119) | 116 (107-130) | 0.27 | 109-120 mmol/L |
| **Sodium (mmol/L)** | 146 (134-154) | 147 (134-153) | 0.86 | 140-150 mmol/L |
| **pH** | 7.33 (7.22-7.39) | 7.31 (7.11-7.39) | 0.12 | 7.33-7.37 |
| **APPLE fast score** | 24 (18-41) | 22.5 (10-40) | 0.55 | |

Legend of table 1: Values are expressed as median (minimum-maximum).

T0, blood sample collected before bolus; HES group; dogs that received a bolus of hydroxyethyl starch 130/0.4; HS group, dogs that received a bolus of hypertonic saline 7.5%; In-TEM, intrinsic thromboelastometry pathway; Ex-TEM, extrinsic thromboelastometry pathway; Fib-TEM, functional fibrinogen; CT, clotting time; CFT, clot formation time; MCF maximum clot firmness;

PT, prothrombin time; aPTT, activated partial thromboplastin time; PCV, microhematocrit, acute patient physiologic and laboratory evaluation fast score (APPLE fast score);

Institutional reference interval for ROTEM parameters are expressed as 95% confidence intervals (Falco et al. 2012).

A value of $P<0.05$ indicates statistically significant differences between HES and HS group.

524 **Table 2:** Altered ROTEM tracings in 3 dogs, before and after bolus administration of

525 hydroxyethyl starch 130/0.4 (HES group).

| | **Dog 4 Hypocoagulable** | | **Dog 7 Hypocoagulable** | | **Dog 8 Hypercoagulable** | | **Institutional reference intervals** |
|---|---|---|---|---|---|---|---|
| ROTEM | **T0** | **T1** | **T0** | **T1** | **T0** | **T1** | |
| **In-TEM** | | | | | | | |
| CT (s) | 140 | 127 | 182 | 223 | 141 | 113 | 126-363 s |
| CFT (s) | 206 | **390** | **368** | **465** | **40** | 47 | 47-224 s |
| MCF (mm) | 50 | **41** | **41** | **39** | 73 | 71 | 50-75 mm |
| α angle (°) | 59 | **42** | **41** | **36** | **82** | 81 | 55-81 ° |
| **Ex-TEM** | | | | | | | |
| CT (s) | **118** | **104** | **169** | **110** | 40 | 34 | 29-92 s |
| CFT (s) | **295** | **463** | **365** | **455** | **44** | 51 | 54-275 s |
| MCF (mm) | 45 | 36 | 39 | 37 | 89 | 76 | 36-73 mm |
| α angle (°) | 53 | **38** | **41** | **42** | **81** | **81** | 47-79 ° |
| **Fib-TEM** | | | | | | | |
| CT (s) | 59 | 85 | 57 | **473** | 37 | 27 | 14-102 s |
| MCF (mm) | **5** | **4** | **5** | **4** | **33** | 23 | 6-26 mm |
| **$MCE_{PLT}$** | 77 | 52 | 59 | 55 | **760** | 287 | 50-235 |
| Standard coagulation | | | | | | | |
| **aPTT (s)** | 12 | 12.5 | 13.5 | **19.8** | 11.2 | 11.8 | 12-16 s |
| **PT (s)** | 8.5 | 9.4 | 9.4 | **11.4** | 8 | 8.6 | 8-10 s |
| **Fibrinogen** | **3.8** [129 | **2.6** [88 | 5.1 [173 | 5.4 [182 | 4.04 [11.9 | 7.8 [267 | 4.4-13.2 (g/L) [150-450 |

| (µmol/L) | mg/dL] | mg/dL] | mg/dL] | mg/dL] | mg/dL] | mg/dL] | mg/dL] |
|---|---|---|---|---|---|---|---|
| **Platelet count (x10$^9$cell/ L)** | **101** (101x10$^3$ cell/µL) | | **88** (88x10$^3$ cell/µL) | | 624 (624x10$^3$ cell/µL) | | 128-543 x10$^3$cell/µL (128-543 x10$^3$cell/µL) |

Legend of table 3: In-TEM, intrinsic thromboelastometry pathway; Ex-TEM, extrinsic thromboelastometry pathway; Fib-TEM, functional fibrinogen; CT, clotting time; CFT, clot formation time; MCF maximum clot firmness; PT, prothrombin time; aPTT, activated partial thromboplastin time.

Bold values are outside the reference interval. Institutional reference interval for ROTEM parameters are expressed as 95% confidence intervals.[9]

545 **Table 3 –** Hemostasis assessment and laboratory parameters of interest at T1.

| **T1** | **HES GROUP** N=13 | **HS GROUP** N=10 | **P value** | **Institutional reference intervals** |
|---|---|---|---|---|
| ROTEM | | | | |
| **In-TEM** | | | | |
| CT (s) | 151 (113-223) | 190 (155-240) | 0.018* | 126-363 s |
| CFT (s) | 120 (47-465) | 104 (57-191) | 0.34 | 47-224 s |
| MCF (mm) | 58 (39-71) | 57 (44-70) | 0.73 | 50-75 mm |
| α angle (°) | 68 (36-81) | 71 (60-78) | 0.38 | 55-81 ° |
| **Ex-TEM** | | | | |
| CT (s) | 46 (26-110) | 42 (37-85) | 0.44 | 29-92 s |
| CFT (s) | 130 (51-463) | 119 (62-148) | 0.46 | 54-275 s |
| MCF (mm) | 58 (36-76) | 58 (52-86) | 0.87 | 36-73 mm |
| α angle (°) | 65 (38-83) | 70 (62-79) | 0.32 | 47-79 ° |
| **Fib-TEM** | | | | |
| CT (s) | 44 (27-473) | 44 (29-78) | 0.88 | 14-102 s |
| MCF (mm) | 10 (4-23) | 11 (7-25) | 0.35 | 6-26 mm |
| **$MCE_{PLT}$** | 128 (52-287) | 121 (101-261) | 0.98 | 50-235 |
| Standard coagulation | | | | |
| **aPTT (s)** | 12.4 (8.5-19.8) | 10.9 (9.8-15) | 0.62 | 12-16 s |
| **PT (s)** | 7.9 | 7.8 | 0.82 | 8-10 s |

| | | | | |
|---|---|---|---|---|
| | (6.4-11.4) | (6.4-9.5) | | |
| **Fibrinogen (µmol/L)** | 6.2 (2.6-7.9) [210 mg/dL (90-270)] | 4.4 (3.2-6.2) [150 mg/dL (110-210)] | 0.021* | 4.4-13.2 (µmol/L) [150-450 mg/dL] |
| Laboratory parameters | | | | |
| **PCV (%)** | 40 (28-48) | 37 (28-42) | 0.025* | 37.5-58.3 % |
| **Total Solid (g/L)** | 0.55 (0.4-0.76) [5.5 g/dl (4-7.6)] | 0.6 (0.4-0.75) [6 g/dl (4-7.5)] | 0.53 | 0.55-0.72 g/L (5.5-7.2 g/dl) |
| **Albumin (g/L)** | 0.23 (0.13-0.32) [2.3 g/dl (1.3-3.2)] | 0.25 (0.16-0.3) [2.5 g/dl (1.6-3)] | 0.73 | 0.3-0.39 g/L (3-3.9 g/dl) |
| **Chloride (mmol/L)** | 115 (90-122) | 129 (109-139) | 0.024* | 109-120 mmol/L |
| **Sodium (mmol/L)** | 145 (134-151) | 154 (139-161) | 0.006* | 140-150 mmol/L |
| **pH** | 7.35 (7.16-7.4) | 7.28 (7.15-7.33) | 0.013* | 7.33-7.37 |

Legend of table 2: Values are expressed as median (minimum-maximum).

T1, blood sample collected after bolus; HES group; dogs that received a bolus of hydroxyethyl starch 130/0.4; HS group, dogs that received a bolus of hypertonic saline 7.5%; In-TEM, intrinsic thromboelastometry pathway; Ex-TEM, extrinsic thromboelastometry pathway; Fib-TEM, functional fibrinogen; CT, clotting time; CFT, clot formation time; MCF maximum clot firmness; PT, prothrombin time; aPTT, activated partial thromboplastin time; PCV, microhematocrit.

Institutional reference interval for ROTEM parameters are expressed as 95% confidence intervals (Falco et al. 2012).

*A value of $P<0.05$ indicates statistically significant differences between HES and HS group.

555 **Figure 1:** Examples of hypocoagulable and normocoagulable thromboelastometric tracings
556 recorded at T0.
557