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

(Article begins on next page)

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1 **Abstract**

2 **Objective** – Compare the impact on hemostasis of intravenous bolus of hydroxyethyl starch

3 130/0.4 (HES) or hypertonic saline 7.5% (HS) in dogs resuscitated for gastric dilation volvulus

4 (GDV).

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

6 **Animals** – 23 client-owned dogs.

7 **Interventions** – Dogs affected by GDV and shock, were randomly assigned to receive HES at 10

8 ml/Kg or HS at 4 ml/Kg per 15 minutes. Blood samples were collected for blood gas analysis,

9 PCV, total protein, albumin, standard coagulation profile and thromboelastometry (ROTEM) at

10 baseline (T0), and at the end of bolus (T1). To assess the differences between the two groups at

11 T1, t-Student test or Wilcoxon rank-sum test were used. To evaluate the differences between T0

12 and T1, ANOVA for paired data or the Wilcoxon matched-pairs signed-ranks test were used. A

13 value of $P<0.05$ was considered for significance.

14 **Measurement and Main Results** – Hemostasis was evaluated by means of prothrombin time,

15 activated partial thromboplastin time, fibrinogen and ROTEM.

16 The study included 13 dogs in HES group and 10 dogs in HS group. Significant differences

17 between groups at T1: increase of clotting time (P=0.018) and decrease in fibrinogen level

18 (P=0.021) in the HS-treated group.

19 Significant differences between T0 and T1: increase of clot formation time (P = 0.046), decrease

20 in maximum clot firmness (P = 0.002) in ex-TEM profile, and decrease of maximum clot

21 firmness (P=0.0117) in fib-TEM profile, for HES group; increase of clotting time (P=0.048) and

22 clot formation time (0.0019), decrease of maximum clot firmness (P=0.031) and α angle

23   (P=0.036) in ex-TEM profile, decrease in α angle (P=0.036) in in-TEM profile, and decrease in

24   maximum clot firmness (P=0.017) in fib-TEM profile, for HS group.

25   **Conclusion –** In dogs affected by GDV, the doses of HES or HS administered have caused

26   few differences in hemostatic analyses, indicating a similar tendency to hypocoagulability.

27

28   **ABBREVIATIONS**

29   ALB: albumin

30   ANOVA: analysis of variance

31   aPTT: activated partial thromboplastin time

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

33   CT: clotting time

34   CFT: clot formation time

35   Ex-TEM: extrinsic thromboelastometry pathway

36   Fib-TEM: functional fibrinogen

37   GDV: gastric dilation and volvulus

38   Hct: hematocrit

39   HES: hydroxyethyl starch

40   HS: hypertonic saline

41   In-TEM: intrinsic thromboelastometry pathway

42   DIC: disseminated intravascular coagulation

43   MCF: maximum clot firmness

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

45 PCV: microhematocrit

46 PT: prothrombin time

47 ROTEM: rotational thromboelastometry

48 TEG: thromboelastography

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

50 T1: blood sample collected after the bolus

51 TP: total protein

52

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

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55

Introduction

57 Intravenous (IV) fluid therapy for resuscitation from cardiovascular shock differs by type of

58 fluid, dosage, side effects, and indications. The two major categories are represented by

59 crystalloid and artificial colloids solutions.[1]

60 Hydroxyethyl starches (HES) are artificial colloid frequently used in veterinary medicine for

61 intravascular volume expansion. In human medicine, adverse effects, as coagulopathies, kidney

62 injury, and tissue storage, are reported after HES administration, which seem to be related to

63 mean molecular weight, molar substitution, and C2/C6 ratio.[2-4] Hemostatic alterations mainly

64 result from hemodilution, but a direct effect of HES macromolecules, such as platelet dysfunction

65 with decreased expression of integrin $\alpha IIb\beta3$, a reduction in clotting factor activities (e.g., factor

66 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

88 profile.[a,28-33] The relative superiority of HES *versus* HS, as a resuscitation fluid for minimizing

89 induced hypocoagulability in dogs with GDV, is unknown.

90 The aim of this study was to compare the impact on hemostasis of fluid resuscitation with an

91 intravenous bolus of either HES (130/0.4) or HS (7.5%) in dogs with GDV, by means of

92 rotational thromboelastometry.

93 Our hypothesis was that HES solution will impair coagulation more than HS solution, leading

94 to a hypocoagulable state which could further complicate the management of dogs

95 hemodynamically unstable.

96

Materials and Methods

Animals

99 The study protocol was approved by the Bioethical Committee of University (protocol number

100 47077 and DL 26/2014, Project 581). This prospective, randomized, multicenter investigation

101 involved client-owned dogs. The owner gave their written, informed consent for participation.

102 All dogs enrolled were patients admitted by Veterinary Teaching Hospital for suspected GDV

103 syndrome based on clinical signs. Inclusion criteria were: diagnosis of GDV based on history,

104 clinical signs, abdominal radiographs, and surgical exploration, and evidence of cardiovascular

105 shock [detected by heart rate >130 bpm, poor pulse quality, capillary refill time > 2 s or < 1 s and

106 venous lactate >2 mmol/L (18 mg/dl)]. Exclusion criteria were: administration of non-steroidal

107 anti-inflammatory drugs, corticosteroid, and artificial colloid or blood products in the 4 weeks

108 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

197 APPLE fast score was 24 (min 18-max 41), 4/13 dogs had gastric necrosis, 3/13 underwent

198 gastrectomy, and 1/13 was euthanized for economic reasons.

199   At T0, the HS-treated group was characterized by: no anemic dog (PCV < 37%), 1/10 was

200 thrombocytopenic [(platelets <128x10^{9}cell/L (<128x10^{3}cell/μL)], 5/10 had an ALB level outside

201 the lower reference range [ALB 0.3 g/L (< 3 g/dl)], and 5/10 had lactate > 6 mmol/L (54 mg/dl).

202 None had PT or aPTT outside the upper reference range; the fibrinogen level was low in 1/10

203 [<4.4 μmol/L (<150 mg/dl)]. (Table1) The median value of APPLE fast score was 22.5 (min 10-

204 max 40), 1/10 dogs had gastric necrosis, 1/10 underwent gastrectomy, and 4/10 were euthanized

205 for economic reasons.

206   The two groups were similar at T0 with no statistically significant difference at baseline for

207 any of the parameters.

208   At T0, most dogs of both groups had ROTEM tracings classified as normal, except 3 dogs in

209 the HES-treated group. In these dogs, hypercoagulability was detected in 1/13 and

210 hypocoagulability in 2/13 dogs. Both hypocoagulable dogs had normal PT, aPTT, and low

211 platelet count, whereas one had a low fibrinogen concentration (Table 2).

212 *Comparison between results at T1*

213   Table 3 presents ROTEM values, standard coagulation profile, and laboratory analysis

214 obtained at T1, and results of comparisons. A statistically significant difference was found

215 between CT (p=0.018) in the in-TEM profiles of the two groups, with an increase in this

216 parameter in the HS-treated group in comparison to HES- treated group.

217   After bolus (T1), a statistically significant decrease was found in fibrinogen level (P=0.021),

218 PCV (P=0.002), pH (P=0.013), and a statistically significant increase was shown in chloride

219 (P=0.024) and sodium (P=0.006); whereas no difference was found between HES and HS-treated

220 groups in MCE_{PLT}, PT, aPTT, TP and ALB (see Table 3).

221 *Comparison between T0 and T1*

222 Statistically significant differences between T0 and T1 in the HES-treated group were:

223 increase in CFT (P = 0.046), decrease in MCF (P = 0.002) in the ex-TEM profile, and decrease in

224 MCF (P=0.0117) in the fib-TEM profile. No difference was found between PT and aPTT

225 concentration, whereas statistically significant decrease in fibrinogen level was observed

226 (P=0.0005).

227 Statistically significant differences between T0 and T1 in the HS-treated group were:

228 increased CT (P=0.048) and CFT (0.0019), and decreased MCF (P=0.031) and α angle (P=0.036)

229 in the ex-TEM profile; decrease in α angle (P=0.036) in the in-TEM profile; decrease in MCF

230 (P=0.017) in the fib-TEM profile, and decrease in MCE_{PLT} (P=0.021). No difference was found in

231 aPTT, whereas there was a statistically significant increase in PT (P=0.0039) and statistically

232 significant decrease in fibrinogen concentration (P=0.027).

233 After HES bolus, a statistically significant decrease was found in PCV (P=0.003), TP

234 (P=0.0005) and ALB (P=0.0002); whereas statistically significant increase was shown in chloride

235 (P=0.0005).

236 After HS bolus, statistically significant decrease was found in PCV (P=0.0001), TP

237 (P=0.0028), and ALB (P=0.0044); whereas statistically significant increase was shown in

238 chloride (P=0.0003), sodium (P=0.0008).

239 *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

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

285   In the HS-treated group, several ROTEM parameters were different between T0 and T1 with a

286   tendency to hypocoagulability, and a decrease in MCE_{PLT} was indicative of reduced platelet

287   contribution to clot contraction/elasticity. Although, no difference was reported at T1 for the

288   MCE_{PLT} between the two groups, and further studies with increase in sample size or with

289   different amount of solutions administered, could help to clarify this result.

290   Hyperosmolarity related to HS can reduces coagulation efficiency, interferes with platelet

291   function and whole blood coagulation, and impairs clotting factors activity, fibrin formation and

292   clot strength.[42-44] Recent in vitro and in vivo veterinary studies have demonstrated a dose-

293   dependent HS effect on canine hemostasis.[13,24,25] In vitro studies have detected impairment of

294   CFT and MCF in the ex-TEM profile of ROTEM analysis, and a recent in vivo study observing a

295   decrease in CT in the fib-TEM profile and a decreased in platelet function, assessed using PFA-

296   100.[13,24,25] Our results are consistent with previous studies, but some differences are present and

297   could be explained by diverse amount of hypertonic crystalloid administered and dog populations

298   selected. Indeed, our dogs were in cardiovascular shock and had hypovolemia, hypoperfusion,

299   and acidosis, conditions that could affect hemostasis.[26,27]

300   At T1, fibrinogen level showed a significant decrease in the HS-treated group (within the

301   reference interval). Interestingly, that result was not associated to change in fib-TEM profile

302   between the two groups, indicating no changes in functional fibrinogen and neglectable clinical

303   relevance. Standard coagulation profile assessment showed no significant changes in PT and

304   aPTT between HS and HES-treated group.

305   Between T0 and T1, fibrinogen level had a significant decrease in both HS and HES-treated

306 groups, whereas PT had a significant increase only in HS-treated group. Similar results on

307 standard coagulation profile were observed by Seshia et al. (2018) after administration of 5

308 ml/Kg of HS over 15 min and 20 ml/Kg of HES over 30 minutes, in healthy dogs, making more

309 likely that these changes were due to HS administration and not exclusive of our population.[45]

310   After the HS bolus administration, a significant increase in sodium and chloride, and a

311 decrease in pH were showed in comparison with HES-treated group, indicating a worsening of

312 acidosis and increase in osmolality, factors that might affected hemostasis in critically ill patients.

313 Those results were expected and explained by the different characteristics of solutions.

314   Regarding the other laboratory parameters evaluated, a significant decrease in PCV, TS and

315 ALB were noted in both groups from T0 to T1, indicating a potential hemodilution effect to both

316 HES and HS administration, although the magnitude of hemodilution appeared greater in the HS-

317 treated group, because PCV at T1 had a significant decrease in comparison with HES-treated

318 group.

319   At baseline (T0), only ROTEM analysis was able to identified two dogs as hypocoagulable in

320 the HES-treated group, whereas this alteration in hemostasis was not detected by PT and aPTT.

321 After the bolus, the ROTEM values worsened and clinical bleeding developed in both dogs

322 (during or after surgery), and the standard coagulation profile at T1 reflected hypocoagulability

323 only in one dog. If ROTEM analysis cannot be perform, physicians should be pay special

324 attention during resuscitation of GDV patient, because dogs could begin hypocoagulable after

325 fluid administration and bleeding. Studies evaluating coagulation in dogs with GDV have

326 reported multiple hemostatic abnormalities at hospital presentation, mainly indicative of

327 hypocoagulability, due to consumption of clotting factors and platelets caused by DIC.[31,33] One

328 abstract published to date has described the use of a viscoelastic technique (TEG) in canine

329 patients with GDV, reporting that dogs with baseline TEG values outside the reference range had

330 higher mortality than dogs without abnormalities.[a]

331   The present study has several limitations. There was no control group treated only with

332 isotonic crystalloids, that would allow to determine the amount of changes in hemostasis due to

333 hemodilution versus a direct effect of HES or HS, and the trial were not blinded.

334   It would have been useful to determine the platelet count also at T1, to identify a decrease in

335 platelet number that could influence with ROTEM parameters, although the MCE_{PLT} assessment

336 allowed for evaluation of platelet contribution.

337   Moreover, the hemostatic changes were evaluated after a bolus of HES or HS, and the effects

338 on coagulation after their redistribution in the extravascular space or after administration of

339 additional fluids are unknown.

340   In conclusion, results obtained in the present study indicate that the doses of HES or HS

341 administered in dogs affected by GDV have determined similar impairment of hemostasis, but

342 the few differences reported in the HS-treated group might suggest a greater effect of this

343 solution.

344   In two dogs ROTEM analysis identified hypocoagulability condition at presentation, which

345 worsened after the bolus and resulted in postoperative clinical bleeding; this status was not

346 detected by the standard coagulation profile performed at T0, highlighting how the ROTEM is a

347 more sensitive tool for the evaluation of coagulation.

348   Further studies are needed to better understand the dose-related effects of HES or HS

349   administration on canine hemostasis and the clinical impact of these alterations.

350

351   **Acknowledgements**

352   The authors declare no conflicts of interest.

353

354   **Footnotes**

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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 (x10⁹cell/L)	168 (88-624) [168x10³cell/µL (88-624)]	239.5 (104-456) [239.5 x10³cell/µL (104-456)]	0.15	128-543 x10⁹cell/L (128-543 x10³cell/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	

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

498   T0, blood sample collected before bolus; HES group; dogs that received a bolus of hydroxyethyl

499   starch 130/0.4; HS group, dogs that received a bolus of hypertonic saline 7.5%; In-TEM, intrinsic

500   thromboelastometry pathway; Ex-TEM, extrinsic thromboelastometry pathway; Fib-TEM,

501   functional fibrinogen; CT, clotting time; CFT, clot formation time; MCF maximum clot firmness;

502 PT, prothrombin time; aPTT, activated partial thromboplastin time; PCV, microhematocrit, acute

503 patient physiologic and laboratory evaluation fast score (APPLE fast score);

504 Institutional reference interval for ROTEM parameters are expressed as 95% confidence intervals

505 (Falco et al. 2012).

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

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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 (x10⁹cell/ L)	**101** (101x10³ cell/µL)		**88** (88x10³ cell/µL)		624 (624x10³ cell/µL)		128-543 x10³cell/µL (128-543 x10³cell/µL)

526 Legend of table 3: In-TEM, intrinsic thromboelastometry pathway; Ex-TEM, extrinsic

527 thromboelastometry pathway; Fib-TEM, functional fibrinogen; CT, clotting time; CFT, clot

528 formation time; MCF maximum clot firmness; PT, prothrombin time; aPTT, activated partial

529 thromboplastin time.

530 Bold values are outside the reference interval. Institutional reference interval for ROTEM

531 parameters are expressed as 95% confidence intervals.[9]

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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

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

547 T1, blood sample collected after bolus; HES group; dogs that received a bolus of hydroxyethyl

548 starch 130/0.4; HS group, dogs that received a bolus of hypertonic saline 7.5%; In-TEM, intrinsic

549 thromboelastometry pathway; Ex-TEM, extrinsic thromboelastometry pathway; Fib-TEM,

550 functional fibrinogen; CT, clotting time; CFT, clot formation time; MCF maximum clot firmness;

551 PT, prothrombin time; aPTT, activated partial thromboplastin time; PCV, microhematocrit.

552 Institutional reference interval for ROTEM parameters are expressed as 95% confidence intervals

553 (Falco et al. 2012).

554 *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