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

Antonio Borrelli*[1], Massimo Giunti[2], Stefano Calipa[2], Angelica Botto[1], Giulio Mengozzi[3], Grassato Lisa[2], Cristiana Maurella[4], Barbara Bruno[1].

[1] University of Turin, Department of Veterinary Science, University of Turin, Largo Paolo Braccini No 2-4, 10095 Grugliasco, Torino, Italy.

[2] University of Bologna, Department of Veterinary Medical Science, Via Tolara di Sopra No 50, 40064 Ozzano dell'Emilia, Bologna, Italy.

[3] University of Turin, Department of Public Health and Pediatric Sciences, C.so Bramante No 88/90, 10100 Torino, Italy.

[4] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, via Bologna No 148, 10154, Torino, Italy.

***Correspondence:** Dr Antonio Borrelli, email: antonio.borrelli@unito.it

University of Turin, Department of Veterinary Science, Largo Paolo Braccini No 2-5, 10095 Grugliasco, Torino, Italy.

Massimo Giunti: massimo.giunti@unibo.it

Stefano Calipa: stefano.calipa2@unibo.it

Angelica Botto: angelica.botto@unito.it

Giulio Mengozzi: giulio.mengozzi@unito.it

Grassato Lisa: lisa.grassato2@unibo.it

Cristiana Maurella: cristiana.maurella@izsto.it

Barbara Bruno: barbara.bruno@unito.it

Abstract

Background: The study's aim was to evaluate, by means of thromboelastometry (ROTEM), the impact on hemostasis of the administration of an intravenous bolus of hydroxyethyl starch 130/0.4 (HES) or hypertonic saline 7.5% (HS) in dogs resuscitated for gastric dilation volvulus.

Results: The dogs were randomly assigned to receive HES at 10 ml/Kg or HS at 4 ml/Kg over 15 minutes. Blood samples were collected at baseline (T0) and at the end of bolus (T1).

The study included 13 dogs in the HES group and 10 dogs in the HS group. There were no statistically significant differences at T0 between the two groups in age, body weight, packed cell volume (PCV), total protein, albumin, standard coagulation profile, platelet number, lactate concentration, Na^+, Cl^- and ROTEM values.

Statistically significant differences between T0 and T1 were as follows: in the HES group, an increase in clot formation time (p=0.046), a decrease in maximum clot firmness (p=0.002) in the ex-TEM profile, and a decrease in maximum clot firmness (p=0.0117) in the fib-TEM profile; in the HS group, an increase in clotting time (p=0.048) and clot formation time (p=0.0019) and a decrease in maximum clot firmness (p=0.031) and α angle (p=0.036) in the ex-TEM profile, a decrease in α angle (p=0.036) in the in-TEM profile, a decrease in maximum clot firmness

(p=0.017) in the fib-TEM profile, and a decrease in platelet contribution to maximum clot elasticity (p=0.021).

At T1, no difference was found between HES and HS groups in the delta percent change of blood volume, PCV, total protein and albumin. A statistically significant increase was identified for maximum clot firmness (p=0.0014) of ex-TEM profile at T1 in the HS group compared with the HES group.

Conclusion: These results indicate that 10 ml/Kg of HES 130/0.4 and 4 ml/Kg of HS 7.5%, administered over 15 minutes, interfere with coagulation but do not cause hypocoagulability in dogs with normal hemostasis at presentation. Decrease in hemostatic efficiency could be due partly to hemodilution, but HES and HS had also direct action on hemostasis: HES acted primarily through fibrinogen impairment, whereas HS decreased platelet contribution to clot strength.

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

Background

Intravenous (IV) fluid therapy for resuscitation from shock differs based on type of fluid, dosage, side effects, and indications. The two main categories are represented by crystalloid and colloid solutions [1].

Hydroxyethyl starches (HES) are artificial colloids with widespread use in veterinary medicine for intravascular volume expansion [2]. Side effects reported after HES administration in humans

include coagulopathies, kidney injury, and tissue storage, which seem to be positively related to mean molecular weight, molar substitution, and C2/C6 ratio [2-4]. Hemostatic alteration more likely results from hemodilution than direct action of HES macromolecules, which causes platelet dysfunction with decreased expression of integrin $\alpha_{IIb}\beta_{3,}$ a reduction in clotting factor activities (e.g., factor VIII and von Willebrand factor), decreased fibrinogen polymerization, and impaired fibrinolysis [5]. Both in vitro and in vivo veterinary studies have investigated hemostatic alterations in dogs after blood dilution with HES, but colloids with different molecular weight, degree of substitution, and dosages have been used. Though many studies have identified a decrease in platelet aggregation and hypocoagulability, often dependent on HES dosage or type, none of them to date have reported clinical bleeding [6-16].

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]. HS administration is associated with benefits and side effects as well: while it may help to reduce endothelial swelling, improves cardiac output, and modulates inflammation, its rapid administration (1 ml/kg/min) may cause bradycardia, hypotension and vomiting [18-21]. Altered coagulation in humans has also been reported [22-23]. Three recent veterinary studies conducted in dogs have shown a decrease in platelet function and hypocoagulability after HS administration [13, 24, 25].

Due to the interaction between inflammation, shock and coagulation, the knowledge of the effects that these solutions on hemostasis of critically ill patients is important to anticipate possible complications and make a monitoring plan [26, 27].

Gastric dilation and volvulus (GDV) is a syndrome causing cardiovascular compromise and systemic alterations that require fluid resuscitation for the initial stabilization phase [28]. The cause of shock is multifactorial: compression of caudal vena cava decreases pre-load, fluids are lost in the gastrointestinal tract, and cardiac arrhythmias, ischemia-reperfusion injury and systemic inflammatory response syndrome could develop [29, 30]. GDV generally affects large-breed dogs that require the administration of many fluids in a short time period, and the use of HES or HS give the technical advantage to expand the intravascular volume using low dosage of fluid, in comparison with crystalloid solutions, facilitating the resuscitation.

Thromboelastometry well represents the cell-based model of coagulation, as it evaluates hemostasis using whole blood, taking into account both the plasma and cellular components. Using this technique, clinicians are better able to identify hypocoagulability and hypercoagulability early on [31, 32, 33, 34]. Three profiles were performed to evaluate: the intrinsic pathway (activation by ellagic acid; in-TEM profile), extrinsic pathway (tissue factor activation; ex-TEM profile), and functional fibrinogen (platelets inactivated with cytochalasin D; fib-TEM profile). For each profile, several parameters are determined: CT represents the first phase of fibrin formation, from activation of the test to clot amplitude of 2 mm; this parameter is mainly affected by the concentration of plasma coagulation factors and coagulation inhibitors (e.g., antithrombin or drugs) [31,32]. CFT expresses the velocity of clot formation and is affected predominantly by platelet count and function and by fibrinogen activity. MCF, the maximum firmness the clot achieves, is determined by both platelet count and function, and fibrin formation in the presence of factor XIII [31,32]. The α angle corresponds to the slope of the tangent on the

elasticity curve; it describes the kinetics of clot formation and is affected mainly by platelet count and function and fibrinogen [31,32].

The aim of this study was to evaluate, by means of thromboelastometry, the impact on hemostasis of the administration of an intravenous bolus of HES (130/0.4) or HS (7.5%) during resuscitation of dogs with gastric dilation volvulus. Our hypothesis was that HES and HS solution could modify coagulation leading to a hypocoagulable state, that in hemodynamically unstable dogs could further worse the management of patients.

Methods

Study Animals

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

All dogs enrolled were patients admitted to the Veterinary Teaching Hospital (University of Turin or University of Bologna) 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 shock (e.g., heart rate >130 bpm, poor pulse quality/hyperdinamic, capillary refill time > 2 s or < 1 s, systolic blood pressure <90 mmHg and venous lactate >2 mmol/L). Exclusion criteria were as follows: administration of nonsteroidal anti-inflammatory drugs, corticosteroid, and artificial colloid or blood products in the 4 weeks preceding the 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 analysis. For each dog, the acute patient physiologic and laboratory evaluation (APPLE) fast-scoring system was calculated at the time of admission to classify illness severity as described by other authors [35]. After application of a catheter in both cephalic veins, blood samples (T0) were collected for CBC (ADVIA 120 Hematology, Siemens Healthcare Diagnostics, USA), biochemical evaluation (ILAB 300 plus, Clinical Chemistry System, Instrumentation Laboratories, Italy), venous blood gas analysis (including electrolytes) (ABL 800 Flex; A. de Mori S.p.A., Italy), packed cell volume, total solids, standard coagulation profile (prothrombin time, activated partial thromboplastin time, fibrinogen) (Coagulometer StART, Diagnostica Stago, USA) and thromboelastometric analysis (ROTEM) (ROTEM, Tem International GmbH, Germany). Intravenous fluid therapy with crystalloids solution (15 ml/Kg per 15 minutes of Ringer lactate) was then administered together with methadone 0.2 mg/Kg IV, and radiographic confirmation of GDV was conducted in the emergency room by portable radiography (right lateral abdominal radiographs).

The dogs were randomly assigned to receive HES 130/0.4 at 10 ml/Kg or HS 7.5% at 4 ml/Kg over 15 minutes. If necessary, percutaneous decompression of the stomach was performed during the bolus. Upon completion of the bolus, whole blood was collected again (T1) for analysis of blood gas, packed cell volume (PCV), total protein (TP), albumin (ALB), standard coagulation profile and ROTEM. To estimate the percentage change in blood volume after the bolus, the formula previously reported by Silverstein et al. (2005) was applied: [(T0 Hct/T1 Hct)– 1] x 100;

where T0 Hct is the PCV before fluid administration and T1 Hct is the PCV at the end of the bolus [36].

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

After the protocol, at the discretion of the attending physician, fluids were administered until the dog was stable enough to be anesthetized and for gastric decompression, gastric lavage and surgery.

Assessment of Hemostasis

Whole blood samples for the coagulation profile 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 that were difficult to obtain (e.g., 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: prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen.

Thromboelastometric analyses were performed according to PROVETS guidelines and the analyses run for 30 minutes [37, 38]. Viscoelastic techniques such as ROTEM analysis measure clot formation kinetics, clot firmness, and rate of dissolution (fibrinolysis) [31, 32]. For each sample, in-TEM, ex-TEM and fib-TEM profiles were. The following parameters were assessed for each profile: clotting time ([CT], s); clot formation time ([CFT], s); maximum clot firmness ([MCF], mm); and α angle (α, °); the profiles are represented as reaction curves. The reference

ranges for these ROTEM parameters were previously established at our institution in 45 healthy dogs [9]. Abnormal ROTEM analysis was defined as more than one ROTEM parameter outside of the maximum or minimum values of our reference interval, in a single profile (Table 1). Changes in parameters that characterize a hypercoagulable trend are a decrease in CT or CFT and an increase in MCF or α angle, whereas an increase in CT or CFT and a decrease in MCF or α angle indicate a trend toward hypocoagulable.

An additional calculated parameter is MCE_{plt} (platelet contribution to maximum clot elasticity), obtained as follows: $MCE_{plt} = MCE_{extem} - MCE_{fibtem}$ [$MCE = (MCF*100)/(100-MCF)$]. [39]

Statistical Analysis

Data were entered in an ad hoc database, analyzed with Stata 14.2 (Stata Statistical Software: Release 11. StataCorp LP, USA), and tested for Normality by a Shapiro-Wilk test. To assess the differences between the two groups at T0, the Student's t-test was performed when data were Normally distributed; otherwise, the Wilcoxon rank-sum test was used. To assess the differences between time T0 and time T1, ANOVA for paired data was used when data were normally distributed; otherwise, the Wilcoxon matched-pairs signed-ranks test was used. To assess the differences between the two groups at each time point, ANOVA for repeated measures or the Friedman test, depending on the data distribution, was also used. A value of $p < 0.05$ was considered significant.

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 1 that died before the end of the 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). The 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 of 20 - max 61). The breeds 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)

ROTEM values and laboratory results parameters of interest are presented in Tables 1 and 2, respectively.

At baseline (T0), in the HES-treated group, 1/13 dogs was anemic (PCV < 37%), 2/13 were thrombocytopenic (platelets <128x10^9/L), 7/13 had an albumin level outside the lower reference range (ALB < 3 g/L), and 5/13 had lactated > 6 mmol/L. None had PT or aPTT outside of the upper reference range; the fibrinogen level was low in 1/13 (< 1.45 g/L) and increased in 1/13 (> 3.85 g/L) (Table 1). The median 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, in the HS-treated group, 0/10 were anemic (PCV < 37%), 1/10 was thrombocytopenic (platelets <128x10^9/L), 5/10 had an albumin level outside the lower reference range (ALB < 3 g/L), and 5/10 had lactated > 6 mmol/L. None had PT or aPTT above the upper reference range; the fibrinogen level was low in 1/10 (< 1.45 g/L) (Table 1). The median 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 dogs were euthanized by intravenous injection of pentobarbital).

There were no statistically significant differences at baseline (T0) between the two groups in terms of age, body weight, PCV, TP, serum albumin concentration, PT, aPTT, fibrinogen level, PLT number, lactate concentration, Na, Cl, APPLE score and ROTEM values.

At T0, dogs of both groups had ROTEM tracings classified as normal, except 3 dogs in the HES-treated group. Hypercoagulability was detected in 1/13 dogs, and hypocoagulability in 2/13 dogs; both hypocoagulable animals had normal PT and aPTT and a low platelet count, and one also had a low fibrinogen level (Table 3).

Comparison between T0 and T1

Table 1 presents the ROTEM values, standard coagulation profile, and platelet count obtained at the two time points (T0 *vs* T1) and the results of comparisons.

Statistically significant differences between T0 and T1 in the HES-treated group were an increase in CFT ($p = 0.046$), a decrease in MCF ($p = 0.002$) in the ex-TEM profile and a decrease in MCF ($p=0.0117$) in the fib-TEM profile. No difference was found between PT and aPTT concentration, neither at baseline nor after the bolus, whereas a statistically significant decrease in fibrinogen level was observed ($p=0.0005$). After HES bolus, statistically significant decreases were found in PCV ($p=0.003$), TP ($p=0.0005$) and albumin ($p=0.0002$), whereas a statistically significant increase was found in Cl ($p=0.0005$) (Table 2).

Statistically significant differences between T0 and T1 in the HS-treated group were increased CT ($p=0.048$) and CFT ($p=0.0019$) and decreased MCF ($p=0.031$) and α angle ($p=0.036$) in the

ex-TEM profile; a decrease in α angle (p=0.036) in the in-TEM profile; a decrease in MCF (p=0.017) in the fib-TEM profile; and a decrease in MCE_{plt} (p=0.021). No difference was found in aPTT before and after the bolus administration, whereas there was a statistically significant increase in PT (p=0.0039) and a statistically significant decrease in fibrinogen concentration (p=0.027). After HS bolus, statistically significant decreases were found in PCV (p=0.0001), TP (p=0.0028), and ALB (p=0.0044), whereas statistically significant increases were shown in Cl (p=0.0003) and Na (p=0.0008) (Table 2).

Comparison between results at T1

After bolus (T1), no difference was found between the HES- and HS-treated groups in terms of the delta percentage change of blood volume, PCV, TP and ALB. A statistically significant difference was found between MCF (p=0.0014) in the ex-TEM profiles of the two groups, with an increase in this parameter in the HS-treated group.

Hypocoagulable ROTEM of two dogs at T1

ROTEM tracings of the two dogs identified as hypocoagulable in the HES group showed a continuous hypocoagulable state after HES administration (T1), with a further decrease in fibrinogen level in dog No 4 and an increase in PT and aPTT outside the reference range in dog No 7 (Table 3). In these dogs, a tendency to bleed was observed during surgery. Postsurgical abdominal bleeding was noted in dog No 4, and the hemorrhage, hemodynamic instability, and coagulopathy were resolved with transfusion of fresh frozen plasma. Dog No 7 experienced bleeding during surgery, followed by epistaxis and hemodynamic instability during recovery from anesthesia. The owners refused further treatments and opted for euthanasia.

Discussion

The present study evaluated the possible negative effects on coagulation of two infusion solutions (HES and HS) administered as a bolus during the resuscitation phase in dogs affected by GDV. Bolus administration produced only minimal changes in ROTEM parameters in the HES-treated group and a trend toward hypocoagulability in the ex-TEM profile for the HS-treated group. According to our definition, no hypocoagulability is present because the mean ROTEM values obtained at T1 remained within the reference interval, but the clinical relevance of detected variations should be further evaluated.

Standard coagulation profile assessment showed no changes in PT and aPTT in the HES-treated group, a statistically significant increase in PT in the HS-treated group (within the reference interval), and a statistically significant decrease in fibrinogen level in both groups (within the reference interval). 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 20 minutes in healthy dogs, making more likely that these changes are due to HS administration and not exclusive of our population [40].

In the HES-treated group, ROTEM results showed a statistically significant increase in CFT and a decrease in MCF in the ex-TEM profile and a decrease in MCF in the fib-TEM profile. These alterations, observed between T0 and T1, indicate a decrease in clot firmness and could be related to a decrease in fibrinogen concentration and platelet function. Changes in the CFT, α angle, and MCF parameters can be influenced by some sample features such as platelet count, fibrinogen concentration, and hematocrit, then the results obtained could be consequent either to direct action of colloid molecules on coagulation, or due to hemodilution [32]. However, since a decrease in MCF in both the ex-TEM and fib-TEM profiles (in the latter, platelets are inhibited

by cytochalasin D) and no changes in MCE_{plt}, implied that fibrinogen impairment is the major determinant of these ROTEM changes.

The MCE_{plt} allows assessing contribution of platelets to clot strength through the comparison between the ex-TEM and fib-TEM tests [41]. After platelets have bound to fibrin via the glycoprotein IIb/IIIa receptor, the clot contracts through the action of cytoplasmic motility proteins inside platelets, such that serum is expelled; clot contractile forces may contribute to clot stiffness. 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.

Other studies have previously evaluated changes in hemostasis following HES 130/0.4 administration using different dosages and sample population of dogs [12, 42, 43]. Reutler et al. (2017) detected a significant increase in in-TEM CFT and a significant decrease in ex-TEM, in-TEM, and fib-TEM MCF after administration of 15 ml/Kg of HES over 30-40 min. Additionally, Gauthier et al. (2015) found a hypocoagulable trend with ROTEG (increase in K and decrease in MA and α angle) after bolus administration of 40 ml/Kg over 30 min. Finally, Seshia et al. (2018) observed a decrease in MCF in the in-TEM and ex-TEM profiles after 20 ml/Kg of HES administered over 20 min. The results obtained in previous studies were similar to ours; however, viscoelastic parameters with a statistically significant change were greater in number in comparison to results obtained by us. This difference probably was related to the different time of infusion and volume of HES used, indeed these factors influence the amount of HES present in the intravascular space and hemodilution. [9] Both the present and previous studies have shown

306 that in dogs, HES administration causes alterations to hemostasis, but the lack of a standard
307 definition of hypocoagulable ROTEM makes interpretation of the results subjective and a
308 comparison among different studies difficult. Remarkably, ROTEM changes reported by
309 previous authors were not associated with clinical bleeding, and most of the coagulation variables
310 were in the reference interval in Gauthier et al. (2015) as well as in the present study.
311 In the HS-treated group, several ROTEM parameters were different between T0 and T1: a
312 statistically significant increase in α angle in the in-TEM profile, a decrease in MCF in the fib-
313 TEM profile, and an ex-TEM profile indicative of hypocoagulability tendency were observed.
314 Moreover, a decrease in MCE_{plt} was indicative of reduced platelet contribution to clot
315 contraction/elasticity. In comparison with the results obtained in the HES-treated group,
316 hemostatic effects induced by HS appeared to be related in particular to a decrease in platelet
317 contribution to clot strength.
318 The supposed effects of HS administration on hemostasis may be related to hyperosmolarity.
319 This characteristic can reduces coagulation efficiency, interferes with platelet function and whole
320 blood coagulation, and impairs clotting factors activity, fibrin formation and clot strength [43, 46,
321 47]. Recent in vitro and in vivo veterinary studies have demonstrated a dose-dependent HS effect
322 on canine hemostasis [13, 24, 25]. In vitro studies have detected impairment of CFT and MCF in
323 the ex-TEM profile of ROTEM analysis, after whole-blood dilution with HS at two different
324 osmolalities (3% and 7.2%), but these studies only partially reflect the effects induced by an in
325 vivo condition because they evaluated a closed and static system [13, 24]. A recent in vivo study
326 assessed whole blood coagulation by means of ROTEM and platelet function using PFA-100 in
327 dogs treated with HS (7.2%) or mannitol [25]. The authors reported that HS decreases platelet

function, and in the ROTEM analysis, observed a decrease only in CT and in the fib-TEM profile. Our results are consistent with previous studies, which have demonstrated that HS solution affects hemostasis, but some differences could be explained by diverse amount of hypertonic crystalloid administered and dog populations selected. Indeed, our dogs were in shock and had hypovolemia, hypoperfusion, and most had acidosis, conditions that could affect hemostasis [26, 27]. However, considering that values obtained in the present study were in the reference interval, these ROTEM findings imply a doubtful clinical effect.

In human patients undergoing elective craniotomy or suffering from traumatic brain injury, in vivo administration of HS caused only minimal changes in ROTEM profiles (increased CFT in the ex-TEM profile and decreased CT in the in-TEM profile) or no changes at all, respectively [44, 45]. The effects on hemostasis of a bolus administration of HS are dose and osmolality dependent, and the results of previous studies could have been affected by the differences in the administered dose (1-3 mg/Kg *vs* 4 ml/Kg) and the lower osmolality of the hypertonic solution used (HS 3%) [44, 45].

Regarding the other laboratory parameters evaluated, a statistically significant decrease in PCV, TP and ALB was noted in both groups at T1, indicating a potential hemodilution effect consequent to both HES and HS administration. The amount of hemodilution appeared similar in the two groups because PCV, TP, ALB and the delta percent change in blood volume were not different at T1. These results imply that the amount of dilutional effect on coagulation could also be similar in the two groups.

In the HES-treated group, ROTEM analysis identified two dogs as hypocoagulable at baseline, whereas this alteration in hemostasis was not detected by PT and aPTT. After the bolus of HES,

ROTEM values worsened, clinical bleeding developed in both dogs (during or after surgery), and the standard coagulation profile reflected hypocoagulability. Studies evaluating coagulation in dogs with GDV have reported multiple hemostatic abnormalities at hospital presentation, mainly indicative of hypocoagulability due to the consumption of clotting factors and platelets caused by DIC [48, 49]. Although researches evaluating sensitivity and specificity of each tool conducted in a large population would be explanatory, ours results already indicate that ROTEM analysis can rapidly identify early the hypocoagulability in comparison with the standard coagulation profile. ROTEM provides a more complete evaluation of coagulation, being a dynamic process, whereas PT and aPTT can only report the time necessary to form fibrin. The authors do not have enough information to determine if the worsened of ROTEM parameters in these two dogs, after resuscitation from shock, was due to hemodilution, HES action or both.

The present study has limitations. The small sample size limits the external validity of the results obtained. In addition, there was no control group treated only with isotonic crystalloids to determine the amount of changes in hemostasis due to hemodilution versus a direct effect of HES or HS. The application of the formula previously used by Silverstein et al. (2005) allowed estimating the hemodilution assessing the percentage change of PCV. Since our dogs were all affected by the same disease and during the resuscitation there were be no bleeding, it could be quite reliable. Using the delta percent change in blood volume to objectify the amount of hemodilution obtained in each group of treatment, has allowed a comparison between infusions administered. Information extrapolated by this parameter and the evaluation of change in PCV, TP and ALB have evidenced no differences in hemodilution between HES and HS group.

It would have been useful to also determine the platelet count at T1 and to identify a decrease in platelet number that could influence ROTEM parameters such as CFT, MCF, and α angle, although the MCE_{PLT} assessment has allowed for evaluation of platelet contribution on clot firmness. Although hemostatic changes were evaluated after a bolus administration of HES or HS, the effects on coagulation are unknown at the end of resuscitation (when the patient are hemodynamically stable), after administration of additional fluids, and after their redistribution in the extravascular space.

Conclusion

In this study, changes in ROTEM parameters reflecting tendency toward hypocoagulability, were detected after bolus administration of HES or HS in dogs with GDV. The number of modified ROTEM variables was higher in the HS-treated group than in the HES-treated group, but all remained in the reference interval. According to our definition, these results seem to 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, but does not cause hypocoagulability. The decrease in hemostatic efficiency could be due partly to hemodilution, but HES and HS had also direct action on hemostasis: HES acted primarily through fibrinogen impairment, whereas HS decreased platelet contribution to clot strength.

Only 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 and that it can identify early hypocagulability conditions.

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

List of abbreviations:

ALB: albumin; APPLE: acute patient physiologic and laboratory evaluation; aPTT: activated partial thromboplastin time; CFT: clot formation time; CT: clotting time; DIC: disseminated intravascular coagulation; GDV: gastric dilation and volvulus; HES: hydroxyethyl starch; HS: hypertonic saline; MCF: maximum clot firmness; MCE_{PLT}: platelet contribution to clot elasticity; PCV: packed cell volume; PT: prothrombin time; ROTEG: thromboelastography; ROTEM: thromboelastometry; TP: total protein.

Declarations

Ethics approval and consent to participate

The protocol was approved by the Bioethics Committee of the University of Turin (protocol number 47077) and Bologna (DL 26/2014, Project 581). The dog owners were informed about the methods and purpose of the study and gave their written informed consent.

Consent to publish

Not applicable.

Availability of data and materials

All data analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AB1 and BB contributed to the conception and design of the study. AB2, BB, GL and SC acquired the data. AB1 and BB interpreted the data. AB1, MG, GL and BB drafted the manuscript. CM performed the statistical analysis. AB2, SC and GM contributed to the conception of the study, participated in its design and coordination, and helped draft the manuscript. All authors read and approved the final manuscript.

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Authors' information

[1] University of Turin, Department of Veterinary Science, Largo Paolo Braccini No 2-4, 10095 Grugliasco, Torino, Italy.

[2] University of Bologna, Department of Veterinary Medical Science, Via Tolara di Sopra No 50, 40064 Ozzano dell'Emilia, Bologna, Italy.

[3] University of Turin, Department of Public Health and Pediatric Sciences, C.so Bramante No 88/90, 10100 Torino, Italy.

[4] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, via Bologna No 148, 10154, Torino, Italy.

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584 **Table 1:** ROTEM analysis and standard coagulation profiles of dogs with gastric/dilation

585 volvulus that received a bolus of hydroxyethyl starch 130/0.4 (10 ml/Kg) or hypertonic saline

586 7.5% (4 ml/Kg) over 15 minutes.

	HES GROUP N=13		**HS GROUP** N=10		**Institutional reference intervals**
ROTEM	**T0**	**T1**	**T0**	**T1**	
In-TEM					
CT (s)	162 (127-365)	151 (113-223)	170 (134-220)	190 (155-240)	126-363 s
CFT (s)	115 (40-368)	120 (47-465)	88 (58-160)	104 (57-191)	47-224 s
MCF (mm)	58 (41-73)	58 (39-71)	62 (50-72)	57 (44-70)	50-75 mm
α angle (°)	68 (41-82)	68 (36-81)	74 (62-79)	71* (60-78)	55-81 °
Ex-TEM					
CT (s)	47 (30-169)	46 (26-110)	40 (30-70)	42* (37-85)	29-92 s
CFT (s)	102 (44-365)	130* (51-463)	85 (56-152)	119* (62-148)	54-275 s
MCF (mm)	62 (39-89)	58* (36-76)	65 (54-81)	58* (52-86)	36-73 mm
α angle (°)	73 (33-83)	65 (38-83)	75 (60-82)	70* (62-79)	47-79 °
Fib-TEM					
CT (s)	51 (28-59)	44 (27-473)	39 (32-73)	44 (29-78)	14-102 s
MCF (mm)	12 (5-33)	10* (4-23)	14 (10-24)	11* (7-25)	6-26 mm
$MCE_{platelet}$	156 (59-760)	128 (52-287)	154 (100-409)	121* (101-261)	50-235
Standard coagulation					

aPTT (s)	12.4 (12-14.2)	12.4 (12.1-19.8)	11.3 (9-15.2)	10.9 (9.8-15)	12-16 s
PT (s)	7.8 (6.1-9.5)	7.9 (6.4-11.4)	6.9 (6.3-9)	7.8* (6.4-9.5)	8-10 s
Fibrinogen (g/L)	2.4 (1.3-4)	2.1* (0.9-2.7)	1.9 (0.5-2.8)	1.5* (1.1-2.1)	1.5-4.50 (g/L)

Values are expressed as medians (minimum-maximum).

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; T0, blood sample collected at presentation, before bolus of hydroxyethyl starch 130/0.4 (10 ml/Kg) or hypertonic saline 7.5% (4 ml/Kg); T1, blood sample collected after 15 minutes of bolus. Institutional reference interval for ROTEM parameters are expressed as 95% confidence intervals [9].

* Indicates statistically significant differences between T0 and T1 ($p < 0.05$).

Table 2: Laboratory parameters of interest assessed in dogs with gastric/dilation volvulus that received a bolus of hydroxyethyl starch 130/0.4 (10 ml/Kg) or hypertonic saline 7.5% (4 ml/Kg) over 15 minutes.

	HES GROUP N=13		**HS GROUP** N=10		**Institutional reference intervals**
	T0	**T1**	**T0**	**T1**	
Packed cell volume (%)	50 (30-55)	40* (28-48)	43.5 (39-51)	37* (28-42)	37.5-58.3%
Platelet count (x 10E09 cell/L)	168 (88-624)		239.5 (104-456)		128-543 x10E09 cell/L
Total Protein (g/L)	0.65 (0.58-0.92)	0.55* (0.4-0.76)	0.74 (0.52-0.89)	0.6* (0.4-0.75)	0.55-0.72 g/L
Albumin (g/L)	0.29 (0.24-0.39)	0.22* (0.13-0.32)	0.3 (0.19-0.34)	0.25* (0.16-0.3)	0.3-0.39 g/L
Chloride (mmol/L)	114 (82-119)	115* (90-122)	116 (107-130)	129* (109-139)	109-120 mmol/L
Sodium (mmol/L)	146 (134-154)	145 (134-151)	147 (134-153)	154* (139-161)	140-150 mmol/L
pH	7.33 (7.22-7.39)	7.35 (7.16-7.4)	7.31 (7.11-7.39)	7.28 (7.15-7.32)	7.33-7.37

Values are expressed as medians (minimum-maximum). T0, blood sample collected before bolus at presentation, before bolus of hydroxyethyl starch 130/0.4 (10 ml/Kg) or hypertonic saline 7.5% (4 ml/Kg); T1, blood sample collected after 15 minutes of 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%.

* Indicates statistically significant differences between T0 and T1 ($p < 0.05$).

615 **Table 3:** Abnormal ROTEM tracings observed in 3 dogs, before and after bolus administration of

616 hydroxyethyl starch 130/0.4 of 10 ml/Kg over 15 minutes (HES group).

	Dog n. 4 Hypocoagulable		**Dog n. 7** Hypocoagulable		**Dog n. 8** Hypercoagulable at T0		**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
$MCE_{platelet}$	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 (g/L)	**1.29**	**0.88**	1.73	1.82	4.04	2.67	1.5-4.50 (g/L)
Platelet count (x 10E09 cell/L)	**101**		**88**		624		128-543 x10E09 cell/L

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. T0, blood sample collected before bolus at presentation, before bolus of hydroxyethyl starch 130/0.4 (10 ml/Kg) or hypertonic saline 7.5% (4 ml/Kg); T1, blood sample collected after 15 minutes of 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%. Hypercoagulable trend: decrease in CT or CFT and increase in MCF or α angle; hypocoagulable trend: increase in CT or CFT, and a decrease in MCF or α angle. Bold values are outside the reference interval. Institutional reference interval for ROTEM parameters are expressed as 95% confidence intervals [9].

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