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Prothrombin and activated partial thromboplastin times, thromboelastography, hematocrit, and platelet count in a feline hemorrhage/over-resuscitation model using lactated Ringer's solution or 6% tetrastarch 130/0.4

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Abstract

Objective: To describe and compare prothrombin time (PT), activated partial thromboplastin time (aPTT), thromboelastography (TEG), HCT, and platelet count measurements in a hemorrhage/over-resuscitation model.

Design: Randomized crossover study.

Setting: University teaching hospital.

Animals: Six cats.

Interventions: Anesthetized cats underwent 3 treatments at 2-month intervals. The treatments were as follows: NHR—no controlled hemorrhage and sham resuscitation; LRS—controlled hemorrhage and lactated Ringer's solution (LRS) for resuscitation; and Voluven—controlled hemorrhage and 6% tetrastarch 130/0.4 for resuscitation. The LRS and Voluven were administered at 60 and 20 mL/kg/h, respectively, for 120 minutes. Blood samples were drawn for PT, aPTT, TEG, HCT, and platelet count measurements at a healthy check (T − 7d), after controlled hemorrhage (T0), at 60 and 120 minutes of resuscitation (T60 and T120), and at 24 hours after completion of resuscitation (T24h). Data were analyzed using a general linear mixed model approach (significance was $P < 0.05$).

Measurements and Main Results: Total median blood loss (controlled hemorrhage and blood sampling from T0 to T120) at T120 was 11.4, 31.0, and 30.8 mL/kg for NHR, LRS, and Voluven, respectively. PT and aPTT during LRS and Voluven were prolonged at T60 and T120 compared to NHR ($P < 0.001$). On TEG, the reaction time, kinetic time,

Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time; TEG, thromboelastography.

and alpha-angle were within reference intervals for cats at all time points in all treatments, while maximum amplitude was less than the reference interval (40 mm) at T0, T60, and T120 during Voluven and at T60 and T120 during LRS compared to NHR (both $P < 0.001$). The HCT and platelet count were significantly lower at T60 and T120 during LRS and Voluven compared to NHR ($P < 0.001$).

Conclusions: Hypocoagulopathy was observed during hemorrhage and liberal fluid resuscitation. Prolongation of PT and aPPT and decreased clot strength may have been caused by hemodilution and platelet loss.

KEYWORDS
activated partial thromboplastin time, feline, prothrombin time, resuscitation, thromboelastography

1 | INTRODUCTION

Cats are often anaesthetized for surgical procedures, and the risk of intraoperative hemorrhage is always present.[1] Intraoperative hemorrhage, if severe enough, might cause hypovolemia and hypotension that may warrant resuscitation using isotonic crystalloid or colloid fluids. Hemodilution in vitro studies on dog blood, where blood has been diluted to standardized HCTs or using various blood:fluid dilution ratios, resulted in coagulopathies.[2,3] Furthermore, reviews of in vivo fluid therapy, in dogs, also highlight clinical coagulopathies as a concern during resuscitation, especially when synthetic colloids, like hydroxyethyl starch, are administered.[4,5] However, the effect of hemorrhage or fluid administration on coagulation in cats is scantily reported[5]; instead, theories are extrapolated from dogs, for which the effects are presumed to be similar. More recently, an in vitro study in which Ringer's acetate and tetrastarch 6% 130/0.42 were mixed with feline fresh whole blood at a ratio of 1:6 and effects on global coagulation were measured using rotational thromboelastometry (ROTEM) reported evidence of hypocoagulability. The tetrastarch 6% 130/0.42 caused a greater hypocoagulable effect compared to the Ringer's acetate fluid. However, the magnitude of the effects from both fluids was mostly within normal reference intervals of the laboratory and therefore the authors expressed reservations about the clinical relevance of these observations.[6]

Screening test methods of hemostasis include determining the prothrombin time (PT) and activated partial thromboplastin time (aPTT).[7] These methods test the functioning of secondary hemostasis only and therefore viscoelastic coagulation testing methods, which assess global coagulation function, have been introduced.[8] Thromboelastography (TEG) is a viscoelastic coagulation testing method in which a tracing is plotted during coagulation, and various variables are determined during the precoagulation, coagulation, and fibrinolysis phases of hemostasis. The screening tests of hemostasis have been used routinely for decades and therefore the preanalytical and analytical methods are established and adhered to in practice.[7] However, TEG is sensitive to many preanalytical and analytical methodological differences and therefore incorrect clinical conclusions could be drawn from the outcomes of this test. Therefore, a standardize testing and reporting protocols should be followed, as set out by PROVETS.[9–14]

Hypocoagulability and hypercoagulability have not yet been clearly defined in cats because there is insufficient evidence to allow formulation of such definitions.[9] The screening assays only detect coagulopathies when there are profound derangements.[7] These screening tests cannot be used to define a hypercoagulable state but may provide information about hypocoagulability.[8,15] The TEG, which assesses global coagulation function, has trace variables that can indicate hypocoagulability, including prolongation in R-times and K-times, and decreases in α-angle, maximum amplitude (MA), and *G*-values compared to laboratory reference intervals (and vice versa for a hypercoagulable state).[9]

Guided by the PROVETS guidelines, the aims of the present study were to investigate the effects of hemorrhage followed immediately by resuscitation with an isotonic crystalloid or hydroxyethyl tetrastarch 6% 130/0.4 on PT, aPTT, TEG, HCT, and platelet count in anaesthetized cats. We hypothesized that there would be no difference in the hemostatic profiles of the cats among the treatments.

2 | MATERIAL AND METHODS

2.1 | Animals

A group of 6 sterilized cats (3 males and 3 females; mean ± standard deviation of 21 ± 1 months and 4.9 ± 1.2 kg) were used for the study. The sample size was based on availability and the study budget. They were part of a colony that was housed in a communal indoor–outdoor cattery. The cats were cared for by experienced research officers managing the cattery and fed a commercial cat diet[a] and offered water ad libitum. The animal ethics committees of the University of Pretoria (v006-15) and University of Witwatersrand (2017-10-68-C-AREC) approved the study. This study was part of a larger fluid resuscitation project where an assessment tool to predict the volume of blood being lost and to identify biomarkers for impending volume overload was primarily investigated for a PhD degree.[1,16,17] Resuscitation fluids were

administered in excess in order to identify biomarkers for impending volume overload. We only report on data relevant to the present study. Four months before starting the project data collection, when the cats underwent neutering, the right common carotid artery was superficialized by suturing the sternohyoid and sternocleidomastoid muscles together underneath the isolated and exposed 1-cm section of the artery.

The cats underwent a health check assessment 1 week prior to the treatments through a clinical examination and hemostatic and hematology profiling. No cats were excluded from participating in the study following the pretreatment evaluation. Food, but not water, was withheld 8 hours prior to treatment.

2.2 | Study procedures

Cats were randomly allocated (balanced single block design using a website[b]) to receive 3 treatments at 2-month intervals. On the morning of treatment, the cat was transferred to the theatre complex of the hospital where it underwent a brief clinical examination and was weighed. The cat was premedicated with buprenorphine hydrochloride[c] (0.02 mg/kg) intramuscularly and left undisturbed for 45 minutes. The cat was then transferred to the anesthetic induction room, and an indwelling catheter[d] was aseptically inserted into one of the cephalic veins and secured. Alfaxalone[e] was administered intravenously by titration to effect to induce general anesthesia. The trachea was intubated with a cuffed endotracheal tube[f], which was secured in place by a gauze roll tied behind the ears, and connected to a pediatric circle breathing system[g]. General anesthesia in the spontaneously breathing cats was maintained by delivering isoflurane[h] in oxygen at a fixed flow rate of 80 mL/kg/min with the vaporizer[i] initially set at 2%. A target end-tidal isoflurane concentration of 1.7% was used as the standard for maintaining general anesthesia throughout the procedures. The ventral neck region was clipped and surgically scrubbed[j] before the cat was transferred to the surgery theatre.

Once in theatre, the cat was placed in dorsal recumbency and connected to the anesthetic delivery device, as previously described. A crystalloid[k] was infused by an electronic device[l] at 5 mL/kg/h via a y-ported administration set[m] connected to the cephalic catheter for peri-anesthetic maintenance fluids (maintenance fluids). Probes and leads were placed and connected to a multiparameter machine[n] to monitor various physiological variables as follows: heart rate by electrocardiogram (ECG), peripheral oxyhemoglobin saturation by pulse oximetry, end-tidal isoflurane concentration and end-tidal carbon dioxide by spectrophotometry gas analysis, respiratory rate by capnography, and esophageal temperature by thermistor probe. Body temperature was maintained within a range of 36.0–38.0°C throughout the procedure by wrapping the cat's body in a blanket. A catheter[o] was inserted into the previously superficialized right carotid artery following skin cut-down to allow for invasive blood pressure monitoring. A catheter° was inserted percutaneously into the left jugular vein for intermittent blood sampling and facilitation of controlled hemorrhage later. Both catheters were inserted by the Seldinger technique.[18] Then, 1 of the 3 randomly allocated treatments was commenced. The treatments were divided into 2 phases, the hemorrhage phase and resuscitation phase, as follows:

Treatment NHR: The cat underwent a sham-controlled hemorrhage phase of 15 minutes duration and a sham resuscitation phase of 120 minutes duration.

Treatment LRS: The cat underwent a controlled hemorrhage phase until an endpoint was reached (see below), followed by a resuscitation phase whereby lactated Ringer's solution[k] was infused at 60 mL/kg/h for 120 minutes.

Treatment Voluven: The cat underwent a controlled hemorrhage phase until an endpoint was reached (see below), followed by a resuscitation phase whereby 6% tetrastarch 130/0.4[p] was infused at 20 mL/kg/h for 120 minutes.

During the hemorrhage phase, blood was drawn manually into a semiclosed system using 20-mL syringes[q] primed with 4 mL of citrate–phosphate–dextrose[r] via the jugular catheter at a targeted rate of 2 mL/kg/min until 1 of 2 endpoints. The endpoint was either a (1) maximum blood drawl of 30 mL/kg or (2) mean arterial blood pressure of <48 mm Hg that persisted for at least 3 minutes, whichever happened first.[1] Blood obtained during the hemorrhage phase was stored for 24 hours in the event that the cat required a transfusion during the recovery phase to treat hemorrhagic shock.

During the resuscitation phase, which started within minutes after the hemorrhage phase, the randomized resuscitation fluid was infused via a second electronic device[l], where its administration set[m] was connected to the y-port of the maintenance fluid administration set. Both fluids were administered simultaneously in LRS and Voluven, and NHR only received maintenance fluids.

Blood samples were obtained and physiological variables were recorded at fixed time points during the hemorrhage and resuscitation phases. On completion of the resuscitation phase, all monitoring equipment and catheters, except the jugular catheter, were removed. The carotid artery catheter was removed, and digital pressure was applied until a stable clot was formed. If a stable clot did not form within 15 minutes, then a hemostatic absorbable mesh[s] was applied using digital pressure for 10 minutes, or until hemostasis was achieved. The cutdown incision site was then sutured using a 2-layer closure technique with absorbable suture material[t]. The cat received a single subcutaneous injection of meloxicam[u] (0.2 mg/kg) and intravenous injection of buprenorphine[†] (0.03 mg/kg). The cat was then transferred to the ICU of the hospital for recovery from general anesthesia and overnight observation without any further treatments or interventions performed. Then, 24 hours later, blood was sampled and the jugular catheter was removed following which the cat was transferred back to the cattery. All cats were rehomed, without evidence of renal or other organ injury or dysfunction, through an adoption process 1 month after conclusion of the data collection phase of the project.

2.3 | Data collection

Data relevant to the present study were collected in the form of coagulation and hematology profiling during the health check (T – 7d), during the treatment period (immediately after hemorrhage phase [T0] and at 60 and 120 min [T60 and T120, respectively]), and 24 hours later (T24h). The total blood loss at each time point was cumulative and included the controlled hemorrhage volume (not applicable in NHR) and all blood samples for profiling. Only the total volumes of fluids that were administered during resuscitation were used for reporting because all cats were administered maintenance fluids during all treatments.

Blood samples relevant to this study were drawn from the jugular vein by direct puncture using a needle and syringe and decanted into 2 different storage tubes[v,w] at T – 7d, or via the jugular catheter into a syringe and immediately decanted into the storage tubes during the treatment period (T0, T60, and T120) and at T24h. Before collection of a blood sample from the catheter, a waste blood sample (1.5 mL) was always discarded. The sodium citrate tube[v] was filled to 2.7 mL, as recommended by the manufacturer to ensure a final sodium citrate concentration 3.2% (109 mmol/L). The stored blood was submitted to the onsite laboratory immediately for handling and measurement of TEG[x], PT[y], aPTT[y], and hematology[z] (hemoglobin concentration, HCT, RBC, and platelet counts) using daily-calibrated machines, operated by experienced veterinary clinical technologists. The RBC and platelet counts were verified on blood smears.

The TEG assay followed a standardized protocol. First, the citrated whole blood was rested for 30 minutes. The cup was prepared by adding 0.02 mL of $CaCl_2$[aa] buffer. Then, 0.4 mL of citrated whole blood was mixed with 0.025 mL of tissue factor[ab] (tissue factor concentration 1:500,040). Then, 0.34 mL of the citrated whole blood mixture was added to the cup, and the assay was started and ran for 90 minutes at 37°C. The remaining citrated whole blood not used in the TEG was then spun down (2500 × *g* for 15 min at room temperature) to separate the cellular components from the plasma. The plasma was then placed into the calibrated machine[y] to measure the PT and aPTT assays using standard manufacture assay methodology. Thromboplastin regent[ac] based on recombinant human tissue factor was used for the for the PT assay, and synthetic phospholipid reagent[ad] was used for the aPPT assay. The clinical pathology laboratory used their healthy cat reference intervals for the control values for the TEG, PT, and aPPT assays for interpretation. Our clinical pathology laboratory adhered to current practice recommendations, and its reference range intervals are similar to those of other cat studies.[19–21]

2.4 | Data analysis

The data were tested for normality by plotting histograms, evaluating descriptive statistics, kurtosis, skewness, and standard error, and performing the Anderson–Darling test for normality. Some of the PT and aPTT results exceeded the upper limit of detection for the assay and therefore these data were rank-transformed prior to further analysis. All variables were compared among treatments and over time, as well as the interaction of time × treatment using a general linear mixed model (fixed factors: time and treatment; random factors: cats) following which variables showing significant differences underwent post hoc comparisons using Bonferroni correction for repeated measures. Model fits were assessed by visually inspecting residual plots to assess linearity, homogeneity of variances, normality, and outliers. Fisher's exact test was used to determine if there was a difference in requiring the hemostatic mesh after arterial catheter removal between NHR treatment to LRS and Voluven, respectively. To aid in interpreting the coagulation assays, selected measured and calculated cardiopulmonary variables were tabulated. Data were nonnormally distributed and therefore reported as median (Q1–Q3) for nonanalyzed variables (blood loss volumes, endpoints reached, volumes of fluid administered, etc), and data analyzed using the general linear mixed model were reported as estimated marginal means with 95% confidence intervals from the models. Data were analyzed using a commercially available software[ae], and statistical significance was set at $P < 0.05$.

3 | RESULTS

The maximum blood draw volume endpoint was applied 4 times (LRS: $n = 2$; Voluven: $n = 2$), while the mean arterial pressure (MAP) endpoint was applied 8 times (LRS: $n = 4$; Voluven: $n = 4$). None of the cats required an autologous blood transfusion to treat hemorrhagic shock at any time during the study. The amount of blood lost at T60 was 7.6 (4.9–7.8), 27.3 (17.1–33.5), and 27.0 (19.2–30.4) mL/kg for NHR, LRS, and Voluven, respectively. The total volume of fluids administered for resuscitation at T60 was 0, 60, and 20 mL/kg for NHR, LRS, and Voluven, respectively. The ratio of fluid administration to blood loss at T60 was approximately 2.2:1 and 0.7:1 for LRS and Voluven, respectively. The total blood lost at T120 was 11.4 (7.4–11.7), 31 (19.4–37.6), and 30.8 (21.6–34.8) mL/kg for NHR, LRS, and Voluven, respectively. The total volume of fluids administered for resuscitation at T120 was 0, 120, and 40 mL/kg for NHR, LRS, and Voluven, respectively. The ratio of fluid administration to blood loss at T120 was approximately 3.9:1 and 1.3:1 for LRS and Voluven, respectively. On removal of the carotid artery catheter, a hemostatic absorbable mesh was required in LRS ($n = 5$; $P = 0.01515$) and Voluven ($n = 3$; $P = 0.1818$) but not NHR. Selected cardiopulmonary variables are presented in Table 1.

Results on hematological variables are summarized in Table S1. The RBC count was significantly reduced during LRS and Voluven at T60 and T120 compared to other time points and also significantly different from the NHR (treatment × time: $P < 0.001$). Hemoglobin concentration and HCT changed in a similar way to the RBC count (both: treatment × time: $P < 0.001$). The platelet count was significantly lower during LRS and Voluven at T60 and T120 compared to other time points and different from NHR (treatment × time: $P < 0.001$). The significantly different values for the hematological variables were lower than the laboratory reference interval for cats (Figure 1).

The PT and aPTT results are summarized in Table S2 and Figure 2. The control reference intervals for PT and aPTT measured by the

TABLE 1 Selected measured and calculated cardiopulmonary and biochemistry variables of 6 anaesthetized cats (buprenorphine, alfaxalone, and isoflurane in oxygen) undergoing controlled hemorrhage and fluid resuscitation using lactated Ringer's solution (LRS) or 6% tetrastarch 130/0.4 (Voluven) or no controlled hemorrhage nor resuscitation (NHR) treatment.

		Time			
		T – 15	T0	T60	T120
Variable	Unit	Median (Q1–Q3)	Median (Q1–Q3)	Median (Q1–Q3)	Median (Q1–Q3)
Treatment NHR					
Heart rate	per min	107 (98–128)	104 (97–128)	144 (109–172)	145 (113–196)
Respiratory rate	per min	16 (9–17)	15 (9–19)	28 (18–32)	31 (19–40)
SAP	mm Hg	89 (81–93)	86 (81–94)	103 (84–125)	95 (83–98)
MAP	mm Hg	68 (61–78)	64 (61–68)	85 (65–102)	72 (63–83)
DAP	mm Hg	54 (47–65)	52 (47–56)	68 (51–82)	58 (49–65)
Lactate	mmol/L	1.4 (1.2–1.8)	1.5 (1.2–1.8)	1.5 (1.3–2.2)	1.9 (1.7–2.2)
BE	mmol/L	−7.8 (−9.0 to −6.5)	−8.1 (−8.9 to −6.5)	−8.0 (−10.4 to −6.5)	−6.9 (−9.3 to −4.8)
Pv_{O_2}	mm Hg	99 (64–113)	93 (73–105)	79 (70–128)	74 (60–93)
Cv_{O_2}	mL/dL	11.1 (10.6–12.0)	10.9 (10.6–11.6)	11.8 (10.5–13.8)	11.3 (10.5–12.0)
OER	%	9.6 (8.3–14.9)	9.6 (8.8–12.8)	11.0 (7.2–14.0)	13.5 (9.4–19.3)
P_{CO_2}(v–a)	mm Hg	50 (39–77)	55 (46–58)	47 (42–86)	41 (22–68)
P_{CO_2}(v–a)/CO_2(a–v)	mm Hg·dL/mL	39.6 (20.8–72.8)	44.4 (32.9–50.8)	29.1 (25.9–93.1)	21.9 (10.0–59.3)
Treatment LRS					
Heart rate	per min	106 (102–119)	165 (151–193)	125 (109–153)	179 (147–206)
Respiratory rate	per min	16 (9–17)	21 (13–25)	17 (9–22)	26 (18–38)
SAP	mm Hg	90 (85–93)	61 (58–71)	92 (87–108)	95 (91–138)
MAP	mm Hg	73 (63–77)	45 (41–49)	71 (64–89)	80 (69–109)
DAP	mm Hg	57 (49–62)	36 (34–42)	55 (48–70)	66 (53–84)

(Continues)

TABLE 1 (Continued)

Variable	Unit	Time			
		T − 15	T0	T60	T120
		Median (Q1–Q3)	Median (Q1–Q3)	Median (Q1–Q3)	Median (Q1–Q3)
Lactate	mmol/L	1.9 (1.6–2.6)	1.8 (1.4–2.3)	2.9 (2.2–4.4)	3.5 (2.8–3.8)
BE	mmol/L	−8.7 (−9.5 to −5.9)	−9.2 (−10.6 to −6.6)	−4.9 (−6.5 to −4.0)	−3.4 (−4.7 to −3.2)
Pvo_2	mm Hg	68 (52–104)	47 (41–57)	101 (76–146)	83 (51–143)
Cvo_2	mL/dL	10.9 (9.3–12.1)	10.5 (9.4–11.1)	6.4 (5.0–6.8)	5.2 (4.4–8.1)
OER	%	14.7 (8.2–24.5)	28.6 (22.4–34.6)	13.1 (11.3–21.3)	15.9 (10.5–27.4)
Pco_2(v–a)	mm Hg	29 (14–56)	13 (7–22)	70 (40–103)	46 (20–108)
Pco_2(v–a)/CO_2(a–v)	mm Hg·dL/mL	15.3 (4.1–49.2)	3.3 (1.4–7.6)	65.2 (33.6–124.0)	58.3 (11.0–108)
Treatment Voluven					
Heart rate	per min	115 (93–123)	143 (131–159)	136 (123–143)	152 (133–202)
Respiratory rate	per min	16 (8–23)	18 (7–19)	27 (8–35)	24 (9–45)
SAP	mm Hg	91 (85–93)	69 (58–74)	99 (93–109)	94 (88–117)
MAP	mm Hg	67 (63–75)	45 (41–48)	75 (69–95)	78 (65–93)
DAP	mm Hg	52 (48–60)	37 (34–39)	56 (52–78)	62 (49–72)
Lactate	mmol/L	1.6 (1.0–2.1)	2.0 (1.4–2.8)	1.2 (0.8–1.3)	1.3 (1.0–1.6)
BE	mmol/L	−7.7 (−9.7 to −5.6)	−8.7 (−10.0 to −7.3)	−6.9 (−8.7 to −5.3)	−7.5 (−8.7 to −4.8)
Pvo_2	mm Hg	82 (65–143)	47 (36–54)	89 (64–150)	103 (87–135)
Cvo_2	mL/dL	10.7 (9.5–12.0)	8.8 (7.9–10.9)	5.6 (5.2–6.5)	4.6 (4.0–5.8)
OER	%	13.3 (7.2–20.0)	30.3 (24.0–44.3)	18.4 (12.3–21.8)	18.1 (13.4–23.14)
Pco_2(v–a)	mm Hg	43 (20–101)	7 (3–20)	57 (29–114)	69 (44–101)
Pco_2(v–a)/CO_2(a–v)	mm Hg·dL/mL	31.2 (9.0–114.2)	1.6 (0.4–6.2)	52.7 (16.9–132.7)	62.9 (44.0–103.5)

Note: Data collected before controlled hemorrhage (T − 15), immediately after hemorrhage but before starting fluid resuscitation (T0), at 60 minutes of fluid resuscitation (T60), and at 120 minutes of fluid resuscitation (T120). Data presented as median (Q1–Q3).

Abbreviations: BE, base excess; Cvo_2, venous content of oxygen; DAP, diastolic arterial blood pressure; MAP, mean arterial blood pressure; OER, oxygen extraction ratio; Pco_2(v–a), venous-to-arterial partial pressure of carbon dioxide gap; Pco_2(v–a)/CO_2(a–v), venous-to-arterial partial pressure of carbon dioxide gap to arterial-to-venous oxygen content ratio; Pvo_2, venous partial pressure of oxygen; SAP, systolic arterial blood pressure.

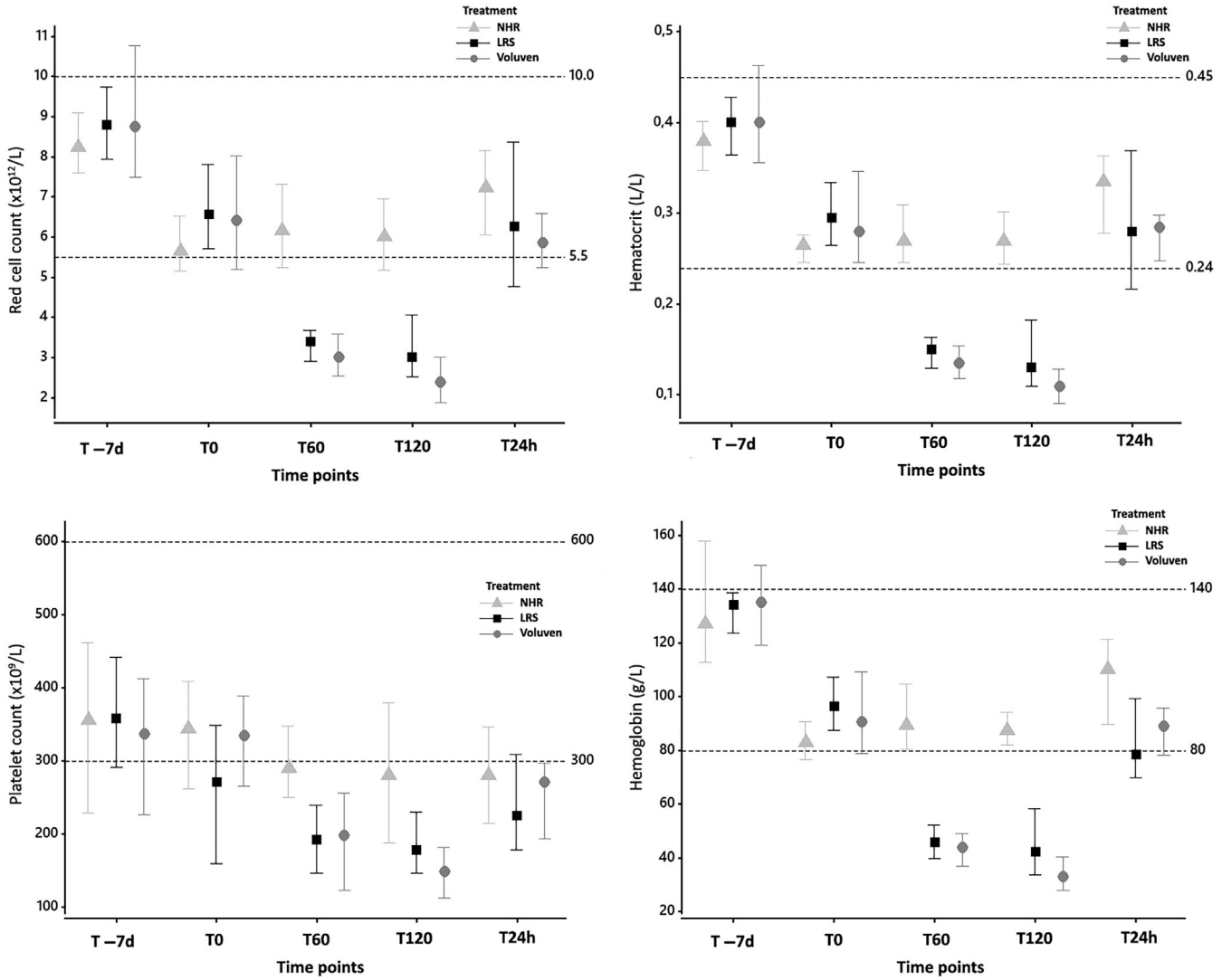


FIGURE 1 Confidence interval plots (95%) of RBC count, platelet count, HCT, and hemoglobin concentration measured over time. Samples were collected at the health check 1 week prior to treatments (T − 7d), immediately after the hemorrhage phase (T0), at 60 and 120 minutes (T60 and T120, respectively) during the resuscitation phase, and 24 hours (T24h) after T0. The cats were anesthetized and underwent 3 treatments, as follows: (1) NHR, with no controlled hemorrhage nor resuscitation; or controlled hemorrhage followed by (2) lactated Ringer's solution for resuscitation (LRS); and (3) 6% tetrastarch 130/0.4 (Voluven) for resuscitation. Cats that underwent a controlled hemorrhage phase, followed by a resuscitation phase with LRS and Voluven administered at 60 and 20 mL/kg/h, respectively, for 120 minutes. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.

laboratory were 10.5 (10.3–10.6) and 11.6 (11.1–11.9) seconds, respectively. The PT was significantly longer during LRS and Voluven at T60 and T120 compared to all other time points but not different among the treatments (time: $P = 0.028$). The aPTT was prolonged during LRS and Voluven at T60 and T120 compared to all other time points and different from NHR (treatment × time: $P < 0.001$).

The TEG values for the measured variables are summarized in Table S3. The R-time did not differ among treatments or over time. Similarly, K-time did not differ among treatments or over time. The α-angle was significantly different over time only in cats receiving the NHR and Voluven treatments, but not LRS (time: $P = 0.013$). Despite the significant differences in α-angle, their median values were all within the laboratory reference intervals for cats (Figure 3). The MA was significantly smaller during LRS at T60 and T120 and during Voluven at T0, T60, and T120 compared to all other time points, but not different among the treatments (time: $P < 0.001$). The G-value varied similarly to the MA and also differed over time, but not among treatments (time: $P < 0.001$). The significant observations in LRS and Voluven over time for the MA and G-value were smaller than the lowest limit of the laboratory reference intervals for cats (Figure 4). There were no differences over time or among treatments for lysis 30 and lysis 60. However, median values and ranges were outside the limits of the laboratory reference intervals for cats after administration of LRS and Voluven over time, compared to NHR (Figure 5).

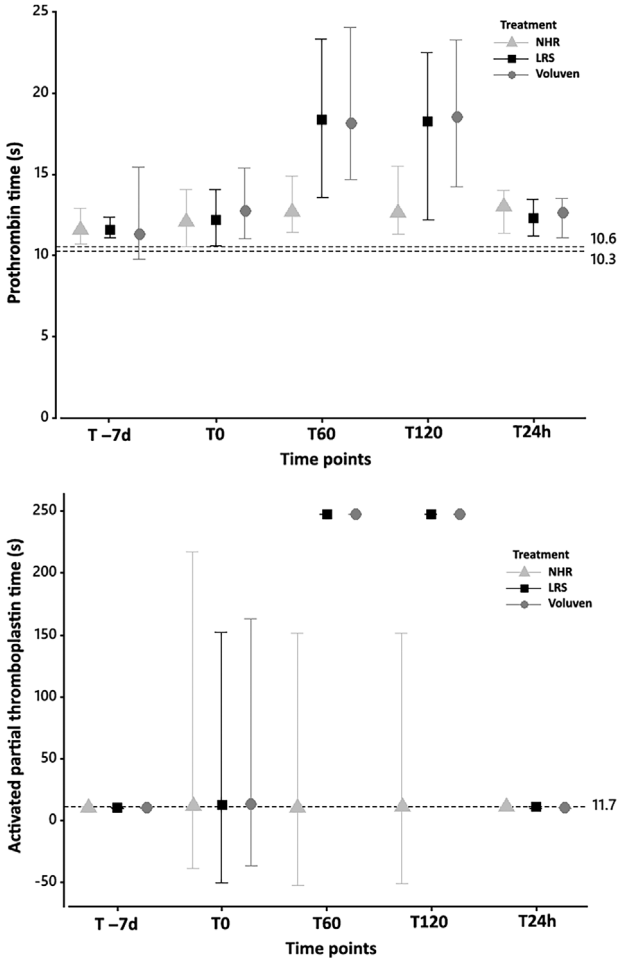


FIGURE 2 Confidence interval plots (95%) of prothrombin time and activated partial prothrombin time. Samples were collected at the health check 1 week prior to treatments (T – 7d), immediately after the hemorrhage phase (T0), at 60 and 120 minutes (T60 and T120, respectively) during the resuscitation phase, and 24 hours (T24h) after T0. The cats were anesthetized and underwent 3 treatments, as follows: (1) NHR, with no controlled hemorrhage nor resuscitation; or controlled hemorrhage followed by (2) lactated Ringer's solution for resuscitation (LRS); and (3) 6% tetrastarch 130/0.4 (Voluven) for resuscitation. Cats that underwent a controlled hemorrhage phase, followed by a resuscitation phase with LRS and Voluven administered at 60 and 20 mL/kg/h, respectively, for 120 minutes. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.

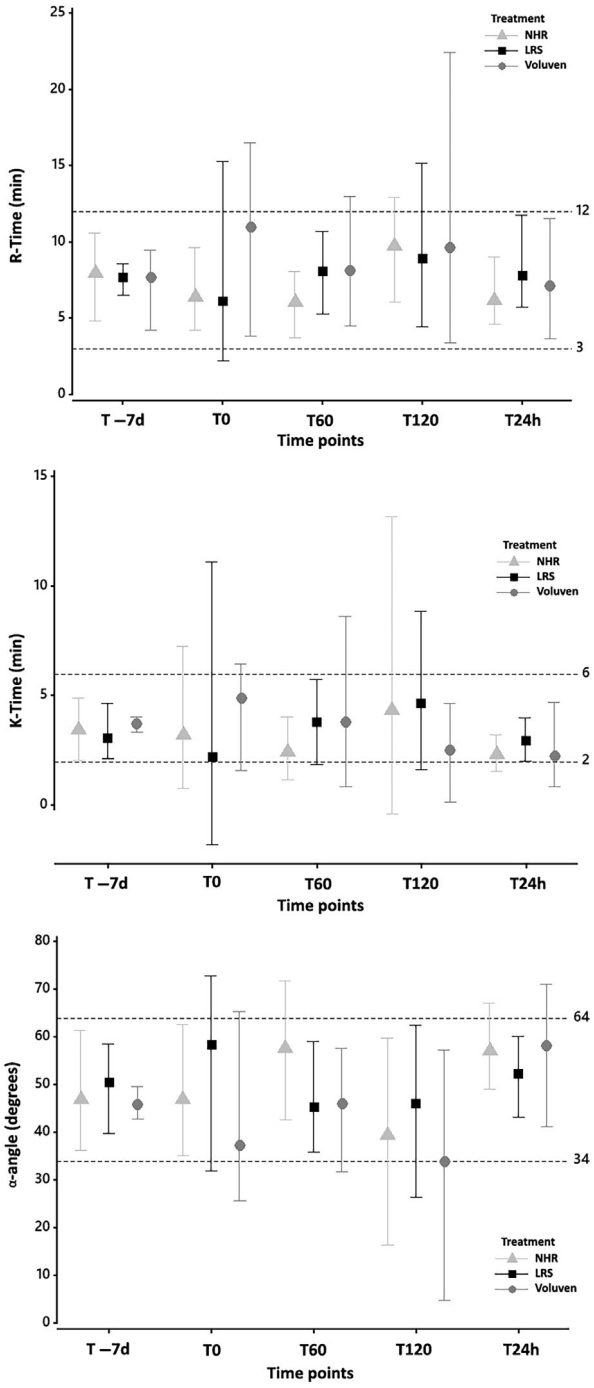
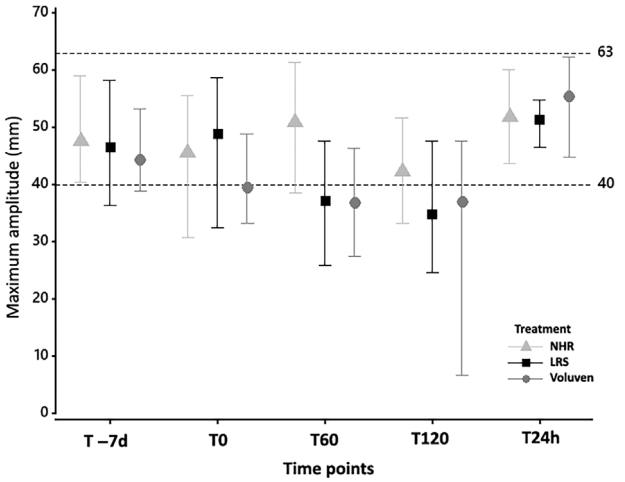


FIGURE 3 Confidence interval plots (95%) of the R-time, K-time, and alpha angle measured by thromboelastography over time. Samples were collected at the health check 1 week prior to treatments (T – 7d), immediately after the hemorrhage phase (T0), at 60 and 120 minutes (T60 and T120, respectively) during the resuscitation phase, and 24 hours (T24h) after T0. The cats were anesthetized and underwent 3 treatments, as follows: (1) NHR, with no controlled hemorrhage nor resuscitation; or controlled hemorrhage followed by (2) lactated Ringer's solution for resuscitation (LRS); and (3) 6% tetrastarch 130/0.4 (Voluven) for resuscitation. Cats that underwent a controlled hemorrhage phase, followed by a resuscitation phase with LRS and Voluven administered at 60 and 20 mL/kg/h, respectively, for 120 minutes. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.

4 | DISCUSSION

Overall, the coagulation profile was normal in the cats during their conscious states (health check and at 24 h after controlled hemorrhage) and after the hemorrhage phase during all treatments. The main observations were somewhat counterintuitive in that the screening assays were prolonged but, unexpectedly, the R-time and K-time of the TEG were within reference intervals. Regardless, all assays detected a state of hypocoagulability in the controlled hemorrhage and resuscitation treatments.



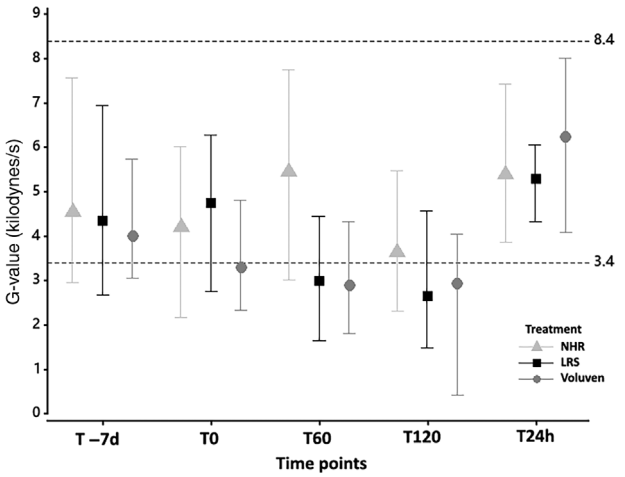
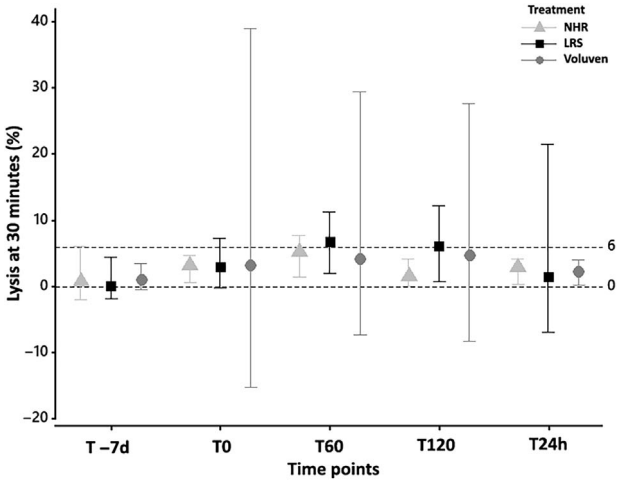


FIGURE 4 Confidence interval plots (95%) of the maximum amplitude and *G*-value measured by thromboelastography over time. Samples were collected at the health check 1 week prior to treatments (T – 7d), immediately after the hemorrhage phase (T0), at 60 and 120 minutes (T60 and T120, respectively) during the resuscitation phase, and 24 hours (T24h) after T0. The cats were anesthetized and underwent 3 treatments, as follows: (1) NHR, with no controlled hemorrhage nor resuscitation; or controlled hemorrhage followed by (2) lactated Ringer's solution for resuscitation (LRS); and (3) 6% tetrastarch 130/0.4 (Voluven) for resuscitation. Cats that underwent a controlled hemorrhage phase, followed by a resuscitation phase with LRS and Voluven administered at 60 and 20 mL/kg/h, respectively, for 120 minutes. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.



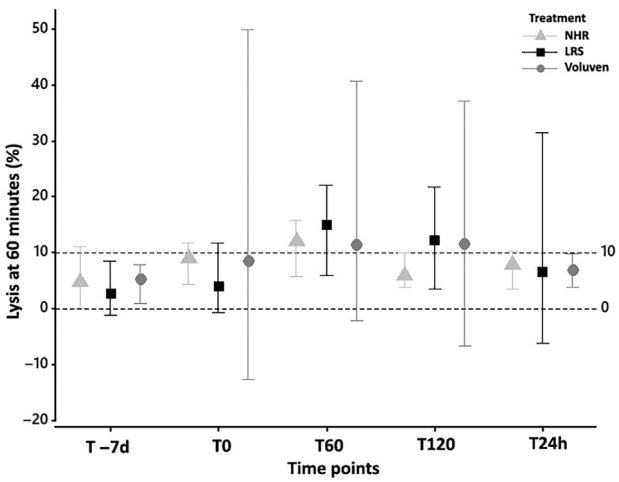


FIGURE 5 Confidence interval plots (95%) of clot lysis at 30 and 60 minutes measured by thromboelastography over time. Samples were collected at the health check 1 week prior to treatments (T – 7d), immediately after the hemorrhage phase (T0), at 60 and 120 minutes (T60 and T120, respectively) during the resuscitation phase, and 24 hours (T24h) after T0. The cats were anesthetized and underwent 3 treatments, as follows: (1) NHR, with no controlled hemorrhage nor resuscitation; or controlled hemorrhage followed by (2) lactated Ringer's solution for resuscitation (LRS); and (3) 6% tetrastarch 130/0.4 (Voluven) for resuscitation. Cats that underwent a controlled hemorrhage phase, followed by a resuscitation phase with LRS and Voluven administered at 60 and 20 mL/kg/h, respectively, for 120 minutes. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.

The prolonged PT and aPTT could have arisen from hemodilution, whereby all coagulation factors were diluted enough to cause the derangement.[4,5] Furthermore, the endothelial glycocalyx degrades after hemorrhage,[22,23] as well as after administering fluids for resuscitation, and this degradation results in vitro prolongation of PT and aPTT.[24] Indeed, the derangements were similar in cats during LRS or Voluven in the present study. The effects of LRS and Voluven were especially profound in the aPTT, a test that assesses the intrinsic and common coagulation pathways.[15] The R-time of the TEG provides a similar measure, but unexpectedly its value remained within the normal reference interval for cats during all treatments at all time points. The R-time has demonstrated correlation to aPTT and especially PT when both assays are initiated using tissue factor, like in our study. Thus, the R-time has been associated with soluble clotting factor activity; however, this correlation, despite being described, has not been repeatable in all studies.[25] Regardless, we expected a prolonged R-time at T60 and T120 during LRS and Voluven treatments. The unexpected difference in these 2 assays could possibly be attributed to the use

of different reagents and activators in aPTT and TEG assays, because if dilution was the only factor, then we would have expected similar findings. It should be noted that the substantial variability in PT, aPTT, and R-times in treatments LRS and Voluven at T60 and T120 can also explain the contradicting discrepancy between these variables. The authors speculate that a larger sample size would potentially mitigate the variability, which can allow for an accurate assessment of agreement between these variables. However, in anemic dogs, the results of viscoelastic tests in coagulation have indicated that they have a hypercoagulability identified by short R-time, short K-time, and increased MA,[2,26] similar to observations of the present study, whereby shorter than expected R-times at T60 and T120 during LRS and Voluven treatments when the HCTs were at their lowest were observed. However, MA, at these time points, was lower than the lowest reference interval level, unlike reports on anemic dogs.[2,26] The low MA observed in the present study could be explained by the decrease in the platelet count at these time points.[25] The platelet counts were corrected and standardized in an in vitro dog study[2] or positively correlated with maximum clot firmness (a thromboelastometry variable similar to MA) in an in vivo dog study.[26] Furthermore, although not measured in our study, the concentration of fibrinogen and its contribution to the fibrin meshwork structure also influence the MA.[26] In our study, we speculate that a dilutional coagulopathy can contribute to low fibrinogen concentration, weak fibrin meshwork structure, and low MA. The low HCT, however, is not the only factor related to a hypercoagulability as overall blood viscosity might also be a factor. The viscosity of whole blood is mainly attributed to the RBC mass under normal physiological conditions, but plasma proteins, especially fibrin, also contribute to viscosity.[2] When there is a low HCT and low blood viscosity, artificial hypercoagulability occurs, while a low HCT and normal viscosity cause hypocoagulability.[2] As with the concern of data variability of the PT and aPTT and R-time data, the other TEG measurements had a large variability, and we speculate that a larger sample size would have mitigated this variability. The fibrinolytic phase of the TEG assay (lysis 30 and lysis 60) is scantly reported on it cats, which makes interpreting this phase of the assays a challenge. In our study, some of the lysis 30 and 60 were above the reference interval at T60 and T120, regardless of treatment. Values of maximum lysis (ROTEM variable) in healthy cats were higher than expected, and this was due to clot retraction rather than true clot lysis and can explain out observation.[27]

Coagulopathies were detected at T60, which translates to total volumes of LRS and Voluven infused at this time point of 60 and 20 mL/kg, respectively. The approximate administration ratios (resuscitation fluid to blood lost volumes) were 2.2:1 and 0.7:1 for LRS and Voluven, respectively, at this time point. The fluid volumes that were administered at this time point were likened to the recommended conventional shock dose rates for cats, but less than the frequently recommended ratios.[28] These findings have potentially far-reaching clinical implications because if the intravascular compartment is resuscitated using these conventional liberal resuscitation guidelines, then coagulopathies can be anticipated. Furthermore, the utilization of a limited-fluid volume resuscitation protocol whereby hypertonic saline (2 mL/kg) alone or in combination with a hydroxyethyl starch (2 mL/kg) is administered after an initial isotonic crystalloid bolus (15–20 mL/kg) may be an alternative therapy for cats unresponsive to conventional fluid therapy protocols.[29] Furthermore, the endothelial glycocalyx is degraded by hemorrhage and by fluid resuscitation regardless of volumes being administered has prompted investigation to find an alternative resuscitation fluid.[22–24] In a rat model, administering fresh frozen plasma improved intravascular volume and restored the endothelial glycocalyx, which suggests fresh frozen plasma is a possible alternative resuscitation fluid.[22] In the present study, cats received very liberal fluid volumes after severe hemorrhage but still had normal coagulation and hematology profiles a day after treatments. Therefore, we speculate that healthy cats have a high fluid tolerance and, unexpectedly, do not require emergency interventions (eg, diuretic treatment) to normalize their total body water and HCT after a hemorrhagic episode.[16] However, cats with subclinical cardiomyopathy may be less fluid tolerant, and screening for cardiac disease is recommended before drafting a fluid plan for resuscitation and maintenance.

The study had notable limitations. The crossover design meant that an atraumatic hemorrhage model was used, and this may limit the translatability of this study to clinical practice where trauma to the vasculature can be associated with natural occurring hemorrhage. Furthermore, it is unknown if this crossover study had a carryover effect of preconditioning the cats to subsequent severe hemorrhage events and hypoxemia. No prehemorrhage blood sample was collected for hemostatic analysis, and this limits the interpretation of distinguishing what effect general anesthesia had on coagulation and hematology from the effect of hemorrhage. The TEG assay used in this study was activated using tissue factor, similar to a PT assay, but future studies should plan to include kaolin-activated TEGs to identify if there is a relationship between R-time and aPTT assays. The blood sampling method used during the health check (needle and syringe) was different from that used during subsequent time points (aspiration from catheter), which is not ideal.[11] However, there were no differences in the tests of coagulation during the health check and the day after treatments, suggesting that the sampling technique did not cause deviations in outcomes of coagulation assays. A complete investigation into the pathophysiology of the derangements (platelet function analysis, factor concentration determination, etc) was not conducted because of study budget constraints. Also, total proteins and fibrinogen concentrations were not measured. Therefore, we cannot state with confidence that other factors, other than hemodilution, do not play a role in the coagulopathies observed in the present study. The sample size was small and thus had the power to reliably detect only large effects. However, the observations herein will assist future researchers in estimating adequate sample sizes to validate these observations under various clinical conditions. The calculated cardiopulmonary variables to aid in quantifying hemorrhagic shock are larger compared to expected ranges of dog hemorrhage models, and this can be because the cats were provided 100% oxygen during the anesthetic, thereby altering arterial to venous gradients compared to breathing room air of 21% oxygen.

5 | CONCLUSIONS

Coagulopathies consistent with laboratory and clinical hypocoagulability were detected during the resuscitation phase in anaesthetized cats after hemorrhage. There were no differences in the coagulopathies between cats that received lactated Ringer's solution and Voluven. Further research is required to identify the mechanisms and pathophysiology responsible for the coagulopathies observed in the present study.

AUTHOR CONTRIBUTIONS

Gareth E. Zeiler, Brighton T. Dzikiti, Peter Kamerman, Roxanne K. Buck, and Andrea Fuller participated in study design. Gareth E. Zeiler, Roxanne K. Buck, and Friederike Pohlin completed the data collection. Gareth E. Zeiler, Brighton T. Dzikiti, Friederike Pohlin, Peter Kamerman, and Andrea Fuller analyzed and interpreted the data. All authors contributed with manuscript drafting and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Notes

[a] Hill's Science Plan Adult Dry Cat Food (chicken or tune flavor), Hill's Pet Nutrition Ltd., Hout Bay, Western Cape.
[b] http://www.jerrydallal.com/random/randomize.htm.
[c] Temgesic 0.3 mg/mL, Reckitt Benckiser Healthcare, Johannesburg, Gauteng.
[d] Jelco I.V. Cathater Radiopaque (22 gauge), Smith Medical International, Johannesburg, Gauteng.
[e] Alfaxan-CD RTU; Afrivet, Midrand, Gauteng.
[f] PVC endotracheal tube (4.0 to 4.5 mm internal diameter), Teleflex Incorporated, Midrand, Gauteng.
[g] Compact paediatric breathing system (15 mm internal diameter), Intersurgical, Midrand, Gauteng.
[h] Isofor; Safeline Pharmaceuticals, Midrand, Gauteng.
[i] EC 5 Isoflurane Vaporiser, Safeline Pharmaceuticals, Midrand, Gauteng.
[j] Biotaine in alcohol, B-Braun, Johannesburg, Gauteng.
[k] Lactated Ringer's Solution, Fresenius Kabi, Midrand, Gauteng.
[l] Infusomat Space, B-Braun, Johannesburg, Gauteng.
[m] Infusomat Space Set, B-Braun, Johannesburg, Gauteng.
[n] Datex-Ohmeda Cardiocap 5; GE Healthcare, Helsinki, Finland.
[o] Arrow arterial catheterization set (22 Gauge, 50 mm), Teleflex Incorporated, Midrand, Gauteng.
[p] Voluven, Fresenius Kabi, Midrand, Gauteng.
[q] Omnifix syringe (20 mL), B-Braun, Johannesburg, Gauteng.
[r] JMS blood bag (450 mL), JMS Singapore, Ang Mo Kio, Central Singapore.
[s] BloodStop iX, Life Science Plus, Palo Alto, CA.
[t] MonoPlus 5/0, B-Braun, Johannesburg, Gauteng.
[u] Petcam; CiplaVet, Midrand, Gauteng.
[v] Sodium citrate 0.109M BD Vacutainers, Becton Dickinson and Company, Plymouth, Devon.
[w] EDTA BD Vacutainers, Becton Dickinson and Company, Plymouth, Devon.
[x] Haemoscope TEG 5000 Thrombelastograph Hemostasis Analyzer, Haemonetics, Braintree, Boston, MA.
[y] ACL Elite, Werfen, Barcelona, Barcelona.
[z] Advia 2120, Siemens Healthineers, Erlangen, Bavaria.
[aa] $CaCL_2$ buffer (0.020 M), HemosIL Instrumentation Laboratory Company, Bedford, MA.
[ab] Dade, Innovin, Siemens Diagnostics, Deerfield, IL.
[ac] RecombiPlasTin 2G, HemosIL Instrumentation Laboratory Company, Bedford, MA.
[ad] SynthAsil, HemosIL Instrumentation Laboratory Company, Bedford, MA.
[ae] MiniTab 18.1, Minitab Inc., State College, PA.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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