Evaluation of the effects of hydroxyethyl starch (130/0.4) administration as a constant rate infusion on plasma colloid osmotic pressure in hypoabluminemic dogs

Abstract

Objective – Evaluate the effects of two constant rate infusions of hydroxyethyl starch (HES) 130/0.4 on plasma colloid osmotic pressure (COP), in hypoalbuminemic dogs.

Design – Cohort prospective study.

Animals – 24 client-owned dogs.

Interventions – Hypoalbuminemic euvolemic dogs [albumin <20 g/L (< 2 g/dl)] with normal perfusion parameters requiring intravenous fluid therapy, were enrolled. In addition to crystalloid, HES 130/0.4 was administered as a constant rate infusion over 24 hours at 1 ml/kg/h (group 1, n=15) or at 2 ml/kg/h (group 2, n=9), in order to support plasma COP. Before infusion, a blood sample was collected to perform cell blood count, serum electrophoresis and serologic tests for some infective diseases. Plasma COP, albumin, packed cell volume and total protein were evaluated serially at baseline (T0) and then at 6, 12 and 24 hours after the start of infusion, and a multilevel model was performed for these parameters to detect statistically significant differences between the two groups.

Measurement and Main Results – Twenty-four dogs were included. No statistically significant differences in COP were found between the two groups; however, a high level of variability has been identified within the single individual. Among the other laboratory analyses, packed cell volume was significantly decreased in group 1 at T12 and T24 compared with T0 ($p<0.001$) and total protein were significantly increased in group 2 at T12 and T24 compared with T0 ($p<0.008$).

Conclusion - No significant effect on plasma COP was found following infusion with HES 130/0.4 at doses of 1 ml/kg/h and 2 ml/kg/h for 24 hours to hypoalbuminemic dogs. The

administered concomitant dose of crystalloids, underlying disease and small sample size were all potential confounding factors.

ABBREVIATIONS

COP: colloid osmotic pressure

CRI: constant rate infusion

HES: hydroxyethyl starch

ICC: intraclass correlation coefficient

PLN: protein losing nephropathy

SIRS: systemic inflammatory response syndrome

Introduction

Colloid osmotic pressure is the pressure exerted by macromolecules across a semipermeable membrane, and it is proportional to the number of molecules present, irrespective of their size.[1] Albumin is the most plentiful protein in the body and accounts for about 60 to 70% of plasma COP, whereas globulins and fibrinogen have a limited effect.[2,3] Conventionally, the Starling equation is used to describe the distribution of fluids from the capillary into the interstitial space as the result of the equilibrium between hydrostatic pressure and plasma COP.[1,4] Accordingly, any condition that increases the hydrostatic pressure or decreases the COP could cause fluid movement into the interstitial space [given a normal filtration coefficient (K_f) and reflection coefficient (σ)]. Some 50 years ago, a protein layer on the luminal surface of the endothelium was discovered: the endothelial glycocalix layer is a web of glycoprotein and

proteoglycans that covers the luminal side of endothelial cells, separating plasma and erythrocytes from the subglycocalyx space. [5,6] The introduction of the concept of subglycocalyx COP, in addition to plasmatic COP, has led to a revision of the Starling equation. [5,6]

Measurement of COP is indicated in dogs treated with colloid solutions or which have low serum albumin, to monitor the efficacy of therapy and reduce complication.[7] In patients with a decrease in intravascular COP fluid therapy poses challenges, because of the risk of further decreasing COP and increasing fluid filtration into the interstitial space, with formation of peripheral edema and effusion. Moreover, the glycocalyx is semipermeable to certain macromolecules such as albumin, and increases of fluid movement to the interstitium may increase the loss of these proteins.[8,9] The diseases associated with a decrease in COP are primarily those that cause a reduction in plasma proteins, particularly albumin. Several equations have been derived from plasma protein concentration to monitor the plasma COP, but because a poor correlation exists between calculated and measured COP, especially in critically ill patients, COP needs to be determined by direct measurement.[10,11]

Artificial colloids are fluids characterized by molecules retained within the vasculature, in the absence of increase vascular permeability, exerting an oncotic pressure.[12] In normal conditions colloid molecules are retained within the vessels longer than crystalloids, resulting in longer-lasting plasma volume expansion, and should contribute to support plasma COP.[12-14] Hydroxyethyl starches (HES) are the most widely used synthetic colloids, and their pharmacological properties (oncotic effect, excretion and half-life) depend on their mean molecular weight, molar substitution, and C2/C6 ratio.[15] The intravascular retention time of the molecules and the oncotic effect, are related to molar substitution and the C2/C6 ratio, which regulate the rate of HES degradation by plasma α-amylase.[16] In human and canine studies, HES

administration has been associated with some side effects such as tissue accumulation, anaphylactic reaction, hypocoagulability and acute kidney injury. [2,17-25]

Few in vivo studies have assessed COP after HES administration in canine patients. [26-29] Two, in particular, have evaluated the effectiveness of HES to increase COP and duration of action in hypoalbuminemic dogs. [26,27] Smiley et al. (1994) noted a significant increase in mean COP after HES 450/0.7 administration (9 to 26 ml/Kg of HES over 6-8 hours), but no relationship between dose and magnitude of increase was found.[26] Moore et al. (1996) found a significant increase in mean COP after a single dose of HES 450/0.7 (7.7 to 43.9 ml/Kg over 6 hours), but the effect disappeared within 12 hours.[27] Other recent studies have obtained contrasting results: Gauthier et al. (2014) have observed an increase in COP after the administration of a bolus of HES 130/0.4, in both healthy dogs and dogs with induced SIRS, whereas Chohan et al. (2011) found no similar increase in healthy anesthetized dogs. [28,29] Administration of a single dose of old generation HES for several hours has resulted in a transient increase in COP, indicating that multiple doses or continuous administration could be necessary to maintain the rise in COP, although these previous studies used different doses of HES and no relationship was found between dose and effects.

In veterinary medicine, the administration of HES 130/0.4 as a CRI, at a rate of 1-2 ml/Kg/h has been reported, but no studies to date have evaluated the efficacy of this protocol to increase COP. [2,17] The aim of the present study was to evaluate the effect of HES 130/0.4 administered as a CRI on plasma COP in hypoalbuminemic dogs. The hypothesis was that administered HES 130/0.4 as a CRI in these dogs would increase the plasma COP.

Materials and Methods

This randomized, clinical prospective study involved client-owned dogs. The protocol was approved by the Bioethics Committee of the author's University, and the owners of all dogs recruited for participation in the study were informed about the study protocol and gave their written consent.

Animals

Among patients admitted to our Veterinary Teaching Hospital for hospitalization, adult dogs (>1 year) with hypoalbuminemia [albumin <20 g/L (2 g/dl)] were recruited if they required intravenous fluid therapy to restore ongoing fluid losses and/or treat dehydration due to their underlying disease (e.g., increased losses, anorexia, and dehydration). Exclusion criteria were: administration of artificial colloid or blood products in the 4 weeks before; history, clinical signs or biochemistry abnormalities indicating the presence of cardiac, pulmonary, renal or liver failure; abnormal perfusion parameters [e.g. heart rate >130 bpm, poor pulse quality, capillary refill time > 2 s or < 1 s, systolic blood pressure <90 mmHg and venous lactate >2 mmol/L (18.02 mg/dl)]; diseases which can change the ratio between albumin and globulin, as the suspicion of multiple myeloma and positive serology for *Ehrlichia canis* and/or *Leishmania infantum*. If further treatments (blood products and/or surgery) were added after T0, the dog was removed from the study.

Study Design

After placement of a catheter in a peripheral vein, fluid therapy with a crystalloid solution (Lactated Ringer's) was calculated based on the percentage of dehydration, ongoing losses and maintenance daily requirement, and the amount was administered in 24 hours. In addition, HES 130/0.4[a] was administered as a CRI (T0) and the dogs were randomly assigned to receive 1 ml/Kg/h or 2 ml/Kg/h of HES for at least 24 hours, by a computer-generated program.[b,2,17] The

pharmacological treatment, prescribed at the start of fluid therapy, remained unchanged during colloid CRI.

Respiratory rate, heart rate, capillary refill time, systolic blood pressure (using Doppler), metatarsal pulse quality and hydration status were evaluated every 4 hours; body weight was obtained every 12 hours.

Samples Collection and Analysis

Before infusion, a blood sample was collected to perform CBC[c] analysis with blood smear evaluation, serum electrophoresis, creatinine,[d] urea,[d] glucose,[d] alkaline phosphatase,[d] aspartate aminotransferase,[d] alanine aminotransferase,[d] γ-glutamyl transpeptidase,[d] fibrinogen level[e] and serological tests for Ehrlichia canis,[f] Borrelia burgdorferi,[f] Anaplasma phagocytophilum,[f] Anaplasma platys,[f] Dirofilaria immitis and Leishmania infantum.[g] After initiation of infusion, plasma COP, albumin,[d] venous blood gas analysis and electrolytes,[h] PCV and total protein were also evaluated at 6 (T6), 12 (T12) and 24 hours (T24). For COP evaluation, whole blood was collected in tubes with lyophilized heparin, and plasma was separated by centrifugation. The plasma COP was measured by means of Osmomat 050,[i] according to manual of instructions. The instrument delivers two consecutive measurements for each sample analyzed; the comparison of both values allows the evaluation of the quality of the measurement.[l]

Statistical analysis

Data were collected and entered in an *ad hoc* database.

The study design estimated an overall sample size of 26 dogs (13 per group), according to the following criteria: Power=80%, Standardized range= 1, Significance level=0.05 and an equal variance between the two groups.

As the Osmomat 050 returns two measurements of the same sample, the ICC was calculated to estimate the reliability of the two measurements. A test for Normality based on skewness, one based on kurtosis and another combining the two tests into an overall test statistic were performed. Levene's robust test statistic was applied to verify the equality of variances. To verify the homogeneity of the two groups at T0, Student's T-test for normally distributed data was performed; otherwise the Wilcoxon rank-sum test was used.

To compare the results obtained from samples collected at the four-time points (T0, T6, T12, T24), a multilevel linear mixed model was used and adjusted for repeated measures, where the random effect was given by the individual and the fixed effect by the time (T0, T6, T12, T24). A similar model was performed to adjust for the disease. Bonferroni adjustment was applied as needed. The Residual Intraclass Correlation for pairs of responses at the individual level of the model was also calculated to verify variability among individuals. The Wilcoxon matched-pairs signed-ranks test was applied to not normally distributed parameters.

All statistics were performed using Stata 14.1.[m] The level of significance was set at $p<0.05$.

Results

Dogs

A total of 30 dogs were enrolled, but only 24 were included to evaluate changes in plasma COP during a constant rate infusion of HES at two dosages: 15 in group 1 (1 ml/kg/h) and 9 in group 2 (2 ml/kg/h). Six dogs were excluded: 2 dogs needed transfusion (albumin or packed red blood cells), 1 dog underwent surgery before protocol completion, 2 samples had technical problems related to the COP measurement, and 1 dog died before protocol completion. Group 1 was composed of 7 males (1 castrated and 6 intact males), and 8 females (2 spayed and 6 intact females), with a median age of 7 years (range 1-12); median body weight was 26.7 Kg (range 5-

39) and the breeds included crossbred (n=8), German Shepherd (n=2), and one each of Miniature Pinscher, Rottweiler, Australian shepherd, Boxer and Border collie.

Group 2 was composed of 3 intact males, and 6 females (2 spayed and 4 intact females), with a median age of 7 years (range 2–10); median body weight was 17.8 Kg (range 5–44) and the breeds included Jack Russell (n=2), crossbred (n=2) and one each of Rottweiler, Labrador retriever, Dachshund, Segugio Italiano, and English bulldog.

In group 1, likely causes of hypoalbuminaemia were: diarrhea in 12/15 (80%) dogs (5/12 (41.6%) with acute diarrhea and 7/12 (58%) with chronic diarrhea), chylothorax in 2/15 (13%) and PLN in 1/15 (6.6%). In group 2, likely causes of hypoalbuminaemia were: diarrhea in 6/9 (66.6%) dogs (1/6 (16.6%) with acute diarrhea and 5/6 (83%) with chronic diarrhea), septic peritonitis in 2/9 (22%) and hypoadrenocorticism in 1/9 (11%). Effusion (excluding a specific cause of effusion such as chylothorax or peritonitis) was noted in 3/15 (20%) in group 1 (abdominal effusion only, transudate) and 2/9 (22%) in group 2 (abdominal effusion only, modified transudate).

Laboratory analysis

The correlation between the two measurements delivered by the Osmomat 050 was very high: ICC=0.995 (CI95% 0.99-1). Table 1 presents the laboratory results for variables of interest and COP values measured at T0, T6, T12, and T24. There were no statistically significant differences in any of the parameters evaluated at T0 between the two groups, and the COP values were below the lower reference range in all dogs (reference range from 17 to 26 mmHg). No statistically significant differences in the COP results were found by the multilevel models nor between the times, neither between the two different doses. A high level of intraindividual variability was identified (figure 1a-b and table 2). No statistically significant differences

between values of the variables at each sampling time were observed even after correction by disease.

In group 1, from T0 to T24: COP was increased in 9/15 dogs (60%), in which only 5/9 dogs presented increase in ALB (55%); COP was decreased in 6/15 dogs (60%), in which only 3/6 dogs presented decrease in ALB (50%). In group 2, from T0 to T24: COP was increased in 2/15 dogs (22%), in which 2/2 dogs presented increase in ALB (100%); COP was decreased in 5/9 dogs (55%), in which only 2/5 dogs presented decrease in ALB (40%); COP and ALB remained unchanged in 2/9 dogs.

A statistically significant decrease in PCV was noted in group 1 at T12 and T24 *versus* T0 ($p<0.001$); a significant increase in total protein was shown in group 2 at T12 and T24 *versus* T0 ($p<0.008$).

Discussion

This study found no significant differences in COP measurements between the two rates of HES 130/0.4 infusion of 1ml/Kg/h and 2 ml/Kg/h administered in hypoalbuminemic dogs over 24 hours; as well as no difference in COP measurements over time. However there was high level of intraindividual variability with both rates of infusion, and each dog responded to the infusion of HES 130/0.4 in an unpredictable way. That variability was influenced by individual factors and could also be attributed to the different amount of crystalloids administered and to the underlying disease status, although adjustments for the specific disease were made, and the results suggested that it did not influence the COP trends (figure 1a-b). The intraindividual variability comes out from the results of the multilevel model: no changes can be attributed to the group (neither increase, nor decrease) but it lies on the individual response to the treatment,

205 independently from the belonging group. This great variability has determined the lack of
206 statistical significance (figure 1a-b).
207 Regarding the other results obtained in this study, a decrease in PCV was observed only in
208 group 1 at T12 and T24. This result could indicate a certain degree of hemodilution, but the
209 concentration of albumin was unchanged and no hemodilution was seen in group 2 which
210 received twice the volume of HES 130/0.4. This unexpected finding could be due to changes in
211 clinical conditions or a difference in the total dose of fluid administered.
212 In group 1, no change in the values of total protein as measured by refractometry was found at
213 any time point , whereas in group 2 an increase was seen only at T12 and T24, though it has been
214 reported that this measurement could be affected by the colloid solutions.[30] Since the refractive
215 index of tetrastarch 130/0.4 is 42 g/L (4.2 g/dl), dilution of blood with colloids could change the
216 refractive index of plasma, but a high dose of infused volume is probably needed to interfere
217 with the refractometer reading.[28,29]
218 Revision of Starling equation has questioned the clinical utility of measuring plasma COP, as
219 the main factor responsible for fluids exchange could be the subglycocalix COP and the integrity
220 of the glycocalyx.[5,6, 31] In the early 2000s, it was discovered that Starling's equation
221 overestimates the effect of interstitial COP on fluid exchange between the intravascular and the
222 interstitial space.[5,31] Above the normal capillary pressure of 20 mmHg, an infusion of colloid
223 solution should increase capillary pressure, raise the volume of filtration to the interstice, but
224 preserve plasma COP; whereas, an infusion of crystalloids should decrease COP and raise the
225 filtration volume more than colloid solutions.[32,33] In our study, although COP did not differ
226 before and after HES administration in our population of hypoalbuminemic dogs, colloid
227 infusion may probably have helped to maintain the COP and the fluid in the intravascular space,

decreasing the rate and amount of fluids lost in the interstitial space. This statement remains a hypothesis, since there was no control group that received only crystalloid infusion in the present study. Moreover, the introduction of the concept of glycocalyx raises the question of the need to restore normal COP value in hypoalbuminemic dogs. Indeed, glycocalyx integrity could be more important than increasing plasma COP and products like pooled albumin, plasma and plasma substitutes could contribute to capillary sealing, rather than acting on the plasma COP. [34,35] Previous studies evaluating the changes in COP after HES administration in dogs obtained different results. Direct comparison with our data would be challenging because of the differences in pathological conditions, type of colloid solutions, and doses and rate of administration.[26-29] Two studies have evaluated the effects of a HES in hypoalbuminemic dogs. Smiley et al (1994) have administered HES 450/0.7 at a dose ranging from 9 to 26 ml/Kg over 6 to 8 hours. They noted a significant increase in mean COP, but no relationship between dose and magnitude of increase.[26] Furthermore, it was reported an improvement in edema or effusion after HES administration. It is difficult to relate this result with an increase intravascular COP, because the revised Starling law states that, since no absorption occurs by the capillaries, filtered fluid returns to the intravascular compartment by the lymphatic vessels.[5,6] Moore et al. (1996) have measured the duration of action of a single dose of HES 450/0.7 (dose ranged from 7.7 to 43.9 ml/Kg) administered over approximately 6 hours, and have found a significant increase in mean COP after HES administration in all dogs, but the effect disappeared within 12 hours after administration.[27] It was also observed that the increase in COP was not significant in the dogs with acute gastrointestinal protein loss, whereas in our study the underlying disease did not appear to influence the COP. This difference could be related to a different studied population: patients in the previous study were mainly affected by canine parvovirus. For this reason, it is

likely that they were puppies or young dogs. Younger animals might differently respond to HES administration.

One study compared the effects of an equal dose of synthetic colloid (40 ml/Kg of HES 130/0.4) or saline, administered over a period of 30 minutes in healthy dogs and dogs with induced SIRS. An increase in COP was observed in both groups treated with HES 130/0.4, with a major increase in the healthy dogs.[29] Interestingly, the rise in COP was greater in the healthy dogs and lasted for 1 to 4 hours, as compared to the 1 to 2 hours noted for the ill dogs. We can hypothesize that, in light of the revised Starling equation, this difference might have been related to an acquired alteration in the glycocalix in the ill dogs, leading to an increase in capillary flow towards the interstitium.[5,34]

Chohan et al. (2011) showed different results after evaluating the administration of HES 600/0.75 or lactated Ringer's (both fluids at a dose of 10 ml/Kg, over 20 minutes) in healthy dogs.[28] At 1 hour post-infusion, a significant decrease in COP was observed in both groups, with significantly lower COP in the lactated Ringer's group than in the HES 600/0.75 group.[28] The explanation could be that the dogs were evaluated during anesthesia and a study of Dismukes et al. (2010) has shown that COP decreases on average 5 mmHg in healthy dogs undergoing general anesthesia.[36]

The different results obtained in aforementioned studies could be related to the dose (bolus) and type of colloid (old generation HES with high molecular weight, high grade of molar substitution and different C2/C6 ratio) administered. We used a new generation HES (130/0.4) administered as a CRI and that could be another reason for the unchanged COP. There aren't pharmacokinetic studies evaluating this type of administration available in dog, but it is known that low molecular weight HES are excreted in greater quantities by renal route.[15] That limits the

amount of HES remaining at the intravascular level influencing COP. It would be interesting to evaluate the effect on COP of HES 130/0.4 administered in hypoalbuminemic dogs as a bolus followed by a CRI. Theoretically, the administration of HES as a CRI, rather than a bolus, might be more indicated in normovolemic and hypoalbuminemic dogs, because the increase in capillary pressure above the normal value increases the volume of transendothelial flow of fluids and proteins with a loss of albumin in the interstitial space.[32,37,38] Despite this, in the current study, the use of HES 130/0.4 as a CRI did not influence significantly COP. Based on previous studies, older generation HES products could possibly be more effective.[26,27]

To the author's knowledge, this is the first study to evaluate the effects of HES 130/0.4, administered as a CRI, on plasma COP in hypoalbuminemic dogs, but some limitations could have affected the generalization of these results. One limitation is the lack of a control group treated with an equal dose of crystalloid to evaluate the trend of COP and confirm whether it decreases during this type of infusion. However, this is a study involving client-owned dogs, and it was considered not ethical to treat some of these patients only with crystalloid, at the time of study design. Another limitation is the small sample size, that could have introduced a Type II error. As set up at the beginning, the study was powered, but there were more exclusions than the expected. Since the total concentration of crystalloid solution, administered for 24 hours, was not recorded, the influence of this variable on our results was not assessed, but no change in the amount of albumin was observed and this is likely to suggest minimal hemodilution, if present.

Other studies are needed to evaluate the advantage of HES 130/0.4 administration as a CRI in hypoalbuminemic dogs relating to the morbidity and the outcome. Moreover, in dogs, the side effects (e.g. hemostatic and/or renal effects) associated with the administration of colloid solutions, have to be taken into account to balance positive effects *versus* the negative ones.

In conclusion, the administration of HES 130/0.4 as a CRI in hypoalbuminemic dogs did not cause significant changes in plasma COP.

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Footnotes

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

b. Microsoft Excel, Redmond, WA, USA.

c. ADVIA 120 Hematology, Siemens Healthcare Diagnostics, Tarrytown, NY, USA.

d. ILAB 300 plus, Clinical Chemistry System, Instrumentation Laboratories, Milan, Italy.

e. Coagulometer StART, Diagnostica Stago, New York, USA.

f. Snap 4 Dx, IDEXX Laboratories, Westbrook, Maine, USA.

g. Snap Leishmania Test, IDEXX Laboratories, Westbrook, Maine, USA.

h. Osmomat 050, Gonotec, GmBH, Berlin, Germany.

i. ABL 800 Flex, A. DE MORI S.p.A., Milano, Italy.

l. Osmomat 050-User guide. Gonotec, GmBH, Berlin, Germany; 2011, p 32.

m. Stata Corp 14.1, Special Edition College Station, Texas, USA

References

1. Hughes D. Transvascular fluid dynamics. Vet Anaesth Analg 2000;27:63-69.

2. Adamik KN, Yozova ID, Regenscheit N. Controversies in the use of hydroxyethyl

starch solutions in small animal emergency and critical care. J Vet Emerg Crit Care 2015; 25 (1):20-47.

3. Fanali G, Di Masi A, Trezza V, et al. Human serum albumin: from bench to bedside. Mol Aspects Med 2012;33(3):209-290.

4.Wellman ML, Di Bartola SP, Kohn W. Applied physiology of body fluids in dogs and cats. In: Di Bartola SP, editor. Fluid, electrolyte and acid-base disorders in small animal practice. 4th ed. St. Louis: Saunders Elsevier; 2011, pp. 2-25.

5. Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. Cardiovasc Res 2010;87(2):198-210.

6. Levick JR. Revision of the Starling principle: new views of tissue fluid balance. J Physiol 2004;557(pt3):704.

7. Waddell LS. Colloid osmotic pressure and osmolality monitoring. In: Silverstein DC, Hopper K., editors. Small animal critical care medicine. 2th ed. St. Louis: Saunders Elsevier; 2015, pp. 978-981.

8. Rieger A. Blood Volume and plasma protein. 3. Changes in blood Volume and plasma proteins after bleeding and immediate substitution with Macrodex, Rheomacrodex and Physiogel in the splenectomized dog. Acta Chirurgica Scandinavica – Supplementum 1967;379:22–38.

9. Vink H, Duling BR. Capillary endothelial surface layer selectively reduces plasma solute distribution volume. Am J Physiol Heart Circ Physiol 2000;278(1):H285–289.

10. Thomas LA, Brown SA. Relationship between colloid osmotic pressure and plasma protein concentration in cattle, horses, dogs, and cats. Am J Vet Res 1992;53(12):2241-2244.

11. Brown SA, Dusza K, Boehmer J. Comparison of measured and calculated values for colloid osmotic pressure in hospitalized animals. Am J Vet Res 1994;55(7):910-915.

12. Hughes D and Boag A. Fluid therapy with macromolecular plasma volume expanders. In: Di Bartola SP, editor. Fluid, electrolyte and acid-base disorders in small animal practice. 4th ed. St. Louis: Saunders Elsevier; 2011, pp. 647-664.

13. Westphal M, James MF, Kozek-Langenecker S, et al. . Anesthesiology 2009;111(1):187-202.

14. Haupt MT, Rackow EC. Colloid osmotic pressure and fluid resuscitation with hetastarch, albumin, and saline solutions. Crit Care Med 1982;10(3):159-162.

15. Jungheinrich C. The starch family: are they all equal? Pharmacokinetics and pharmacodynamics of hydroxyethyl starches. Trans Altern Trans Med 2007(3);9:152-163.

16. Kozek-Langenecker A, Scharbert G. Effects of hydroxyethyl starches on hemostasis. Trans Altern Trans Med 2007;9(3):173-181.

17. Glover PA, Rudloff E, Kirby R. Hydroxyethyl starch: a review of pharmacokinetics, pharmacodynamics, current products, and potential clinical risks, benefits, and use. J Vet Emerg Crit Care 2014;24(6):642-661.

18. Hayes G, Benedicenti L, Mathews K. Retrospective cohort study on the incidence of acute kidney injury and death following hydroxyethyl starch (HES 10% 250/0.5/5:1) administration in dogs (2007-2010). J Vet Emerg Crit Care 2016;26(1):35-40.

19. Smart L, Jandrey KE, Kass PH, et al. The effect of hetastarch (670/0.75) in vivo on platelet closure time in the dog. J Vet Emerg Crit Care 2009;19(5):444–449.

20. Chohan AS, Greene SA, Grubb TL, et al. Effects of 6% hetastarch (600/0.75) or lactated Ringer's solution on hemostatic variables and clinical bleeding in healthy dogs anesthetized for orthopedic surgery. Vet Anaesth Analg 2011;38(2):94–105.

21. Gauthier V, Holowaychuk MK, Kerr CL, Bersenas AME, Darren Wood R. Effect of

366 synthetic colloid administration on coagulation in healthy dogs and dogs with systematic
367 inflammation. J Vet Intern Med 2015;29(1):276- 285.
368 22. McBride D, Hosgood G, Raisis A, Smart L. Platelet closure time in anesthetized Greyhounds
369 with hemorrhagic shock treated with hydroxyrthyl starch 130/0.4 or 0,9% sodium chloride
370 infusion. J Vet Emerg Crit Care 2016;26(4):509-515.
371 23. Helmbold KA, Mellema MS, Hoper K, Epstein SE. The effect of hetastarch 670/0.75
372 administered in vivo as a constant rate infusion on platelet closure time. J Vet Emerg Crit Care
373 2014;24(4):381-387.
374 24. Reutler A, Flammer SA, Howard J, Adamik KA. Comparison of the effects of a balanced
375 crystalloid-based and saline based tetrastarch solution on canine whole blood coagulation and
376 platelet function. J Vet Emerg Crit Care 2017; 27(1): 23-34.
377 25. Botto A, Bruno B, Maurella C, et al. Thromboelastometric assessment of hemostasis
378 following hydroxyethyl starch (130/0.4) administration as a constant rate infusion in
379 hypoalbuminemic dogs. BMC Vet Res. 2018 Jan 31;14(1):33
380 26. Smiley LE, Garvey MS. The use of hetastarch as adjunct therapy in 26 dogs with
381 hypoalbuminemia: a phase two clinical trial. J Vet Intern Med 1994;8(3):195-202.
382 27. Moore LE, Garvey MS. The effect of hetastarch on serum colloid oncotic pressure in
383 hypoalbuminemic dogs. J Vet Intern Med 1996;10(5):300-3.
384 28. Chohan AS, Greene SA, Grubb TL, et al. Effects of 6% hetastarch (600/0.75) or lactated
385 Ringer's solution on hemostatic variables and clinical bleeding in healthy dogs anesthetized for
386 orthopedic surgery. Vet Anaesth Analg 2011;38(2):94-105.

29. Gauthier V, Holowaychuk MK, Kerr CL, et al. Effect of synthetic colloid administration on hemodynamic and laboratory variables in healthy dogs and dogs with systemic inflammation. J Vet Emerg Crit Care 2014;24(3):251-258.

30. Bumpus SE, Haskins SC, Kass PH. Effect of synthetic colloids on refractometric readings of total solids. J Vet Emerg Crit Care 1998;8(1):21-26.

31. Schött U, Solomon C, Fries D, et al. The endothelial glycocalyx and its disruption, protection and regeneration: a narrative review. Scand J Trauma Resusc Emerg Med 2016;24:48.

32. Adamson RH, Lenz JF, Zhang X, et al. Oncotic pressures opposing filtration across non-fenestrated rat microvessels. J Physiol 2004;557(pt 3):889-907.

33. Clough G. Relationship between microvascular permeability and ultrastructure. Prog Biophys Mol Biol 1991;55(1):47-69.

34. Woodcock TE, Woodcock TM. Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy. Br J Anaesth 2012;108(3):384-394.

35. Jacob M, Rehm M, Loetsch M, et al. The endothelial glycocalyx prefers albumin for evoking shear stress-induced, nitric oxide-mediated coronary dilatation. J Vasc Res 2007; 44(6):435-443.

36. Dismukes DI, Thomovsky EJ, Mann FA et al. Effects of general anesthesia on plasma colloid oncotic pressure in dogs. J Am Vet Med Assoc 2010;236(3):309-311.

37. Berg S, Golster M, Lisander B. Albumin extravasation and tissue washout of hyaluronan after plasma volume expansion with crystalloid or hypooncotic colloid solutions. Acta Anaesthesiol Scand 2002;46(2):166-172.

38. Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. Br J Anaesth 2000;85(4):599-610.

39. Kudryk B, Okada M, Redman CM, et al. Biosynthesis of dog fibrinogen. Characterization of nascent fibrinogen in the rough endoplasmic reticulum. Eur J Biochem. 1982;125(3):673-682.

Table 1- Laboratory analysis for variables of interest and COP values measured at T0, T6, T12 and T24 (Group 1 N=15; Group 2 N=9).

Group 1 [1 ml/kg/h]				
Variable	**T0**	**T6**	**T12**	**T24**
COP (mmHg)	9.2 (7-10.8)	9.3 (7.5-11.1)	9.3 (7.3-11.1)	8.9 (7.4-10.8)
Packed cell volume (%)	40 (37-45)	39 (36-44)	37 (34-42)*	39 (34-42)*
Total protein (g/L)	33 (30-44) [3.3 (3-4.4) g/dl]	35 (30-44) [3.5 (3-4.4) g/dl]	37 (30-40) [3.7 (3-4) g/dl]	42 (30-44) [4.2 (3-4.4)g/dl]
Albumin (g/L)	16 (16-18) [1.6 (1.6-1.8) g/dl]	16 (15-19) [1.6 (1.5-1.9) g/dl]	16 (15-18) [1.6 (1.5-1.8) g/dl]	16 (14-20) [1.6 (1.4-2)g/dl]
Fibrinogen (µmol/L)	13 (6.8-34.5) [443 (232-1174) mg/dl]			
Group 2 [2 ml/kg/h]				
Variable	**T0**	**T6**	**T12**	**T24**
COP (mmHg)	9.5 (7.9-11.7)	8.4 (7.7-9.6)	9.7 (7.3-10.1)	8.6 (7.3-10.2)
Packed cell volume (%)	38 (33-42)	34 (31-36)	35 (31-44)	34 (29-39)
Total protein (g/L)	32 (28-37) [3.2 (2.8-3.7) g/dl]	35 (28-39) [3.5 (2.8-3.9) g/dl]	36 (26-37) * [3.6 (2.6-3.7) g/dl]	35 (32-42) * [3.5 (3.2-4.2) g/dl]
Albumin (g/L)	15 (14-15) [1.5 (1.4-1.5) g/dl]	15 (13-15) [1.5 (1.3-1.5) g/dl]	15 (13-16) [1.5 (1.3-1.6) g/dl]	14 (13-17) [1.4 (1.3-1.7)g/dl]
Fibrinogen (µmol/L)	9.3 (6.6-21.8) [318 (223-741) mg/dl]			

Legend of table 1: Data are reported as median (range, 25^{th}-75^{th} percentile). Group 1, CRI at 1 ml/kg/h of HES 130/0.4 administered in hypoalbuminemic dogs, Group 2, CRI at 2 ml/kg/h of HES 130/0.4 administered in hypoalbuminemic dogs, COP, colloid osmotic pressure. The institutional reference interval for COP ranges from 17 to 26 mmHg. Fibrinogen level was measured at T0 because marked increase can influence COP; it was not repeated because it changes very slowly over time.[39]

* statistically significant difference between T0 and T12 and between T0 and T24, $p<0.05$

Table 2- Part of the multilevel model showing the variability within the dogs.

Random-effects parameters	Estimate	95% CI	
Individual variance	5.21	2.89	9.38
Residual variance	0.82	0.59	1.13

On a total of about 6 of variance, 5.21 was attributable to the single dog, and just a minor part of variance was due to the differences among the two groups and among the times (0.82).

Figure 1

Figure 1a: Trend of COP values measured at different sampling times in each dog of group 1

Legend: COP, colloid osmotic pressure; Group 1, CRI at 1 ml/kg/h of HES 130/0.4 administered in hypoalbuminemic dogs; T0, baseline; T1, 6 hour after the start of CRI; T2, 12 hour after the start of CRI; T3, 24 hour the after start of CRI.

Figure 1b: Trend of COP values measured at different sampling times in each dog of group 2

Legend: COP, colloid osmotic pressure; Group 2, CRI at 2 ml/kg/h of HES 130/0.4 administered in hypoalbuminemic dogs; T0, baseline; T1, 6 hour after the start of CRI; T2, 12 hour after the start of CRI; T3, 24 hour the after start of CRI.