A comparison of a 4% modified fluid gelatin and a 6% hydroxyethyl starch on haemodilution, colloid osmotic pressure, haemostasis and renal parameters in healthy ponies

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Submitted in partial fulfilment of the requirements for an MMedVet degree in the Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, South Africa.

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TABLE OF CONTENTS

DECLARATION

I hereby declare that this dissertation, submitted for the degree MMedVet (Equine Medicine), in the Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, is my own work and has not been submitted to another university for a degree, and that the data in this dissertation are the results of my own investigations.

Zoë Ford

12th November 2015

ETHICS STATEMENT

I Zoë Ford, the author, have obtained the applicable research ethics approval for the research described in this work. I declare that I have observed the ethical standards required in terms of the University of Pretoria's Code of ethics for researchers and the policy guidelines for responsible research.

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ABBREVIATIONS

aPTT	Activated partial thromboplastin time
CBC	Complete blood count
CI	Confidence interval
COP	Colloid osmotic pressure
DIC	Disseminated intravascular coagulation
EDTA	Ethylenediaminetetraacetic acid
GGT	Gamma glutamyltransferase
HES	Hydroxyethyl starch
MFG	Modified fluid gelatin
mRNA	Messenger ribonucleic acid
MWw	Weight-average molecular weight
PT	Prothrombin time
RR	Relative risk
TEG	Thromboelastography
TSP	Total serum protein
UGC	Urine gamma glutamyltransferase to creatinine ratio
UPC	Urine protein to creatinine ratio
USE	Urine sediment examination

USG	Urine specific gravity
vWF	von Willebrand factor
VASP	Vasodilator-stimulated phosphoprotein

LIST OF TABLES

LIST OF FIGURES

SUMMARY

A comparison of a 4% modified fluid gelatin and a 6% hydroxyethyl starch on haemodilution, colloid osmotic pressure, haemostasis and renal parameters in healthy ponies

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Degree: MMedVet (Equine Medicine)

Reasons for performing study: Adverse effects on renal health and haemostasis have been documented in human patients administered hydroxyethyl starches (HESs). Gelatins could provide useful substitutes should similar adverse effects be identified in horses.

Objectives: To compare the effects of a 4% modified fluid gelatin (MFG) with a 6% (130/0.4) HES on haemodilution, colloid osmotic pressure (COP), haemostasis and renal parameters in healthy ponies.

Study Design: Randomised crossover experiment.

Methods: Three treatments (A=10 ml/kg bwt HES, B=10 ml/kg bwt MFG and C=20 ml/kg bwt MFG) were administered to 6 healthy ponies with a 1-week washout period. Haematocrit, platelet count, total serum protein, COP, thromboelastography (TEG), prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen were measured at baseline and at multiple time points up to 24 h post-infusion. Serum creatinine, urine specific gravity (USG), urine protein: creatinine ratio (UPC), urine gamma glutamyltransferase:creatinine ratio (UGC) and urine sediment examination (USE) were performed before and 24 h after each treatment, as well as one week after the final treatment.

Results: All treatments resulted in significant haemodilution and increases in COP, with treatment C having a significantly greater effect on haematocrit than other treatments. The platelet count decreased with all treatments and was significantly lower following treatment C compared with treatment B. No clinically relevant differences were observed in any of the TEG parameters within or between treatments. No significant differences in PT, aPTT or fibrinogen were observed between treatments. Serum creatinine, UPC and UGC did not change significantly between pre- and post-study measurements. USG and USE remained within normal limits.

Conclusions: MFG could be considered as an alternative to HES for volume expansion and oncotic support. Neither MFG nor HES were associated with clinically significant adverse effects on haemostasis or renal parameters.

Key terms: Colloid, gelatin, hydroxyethyl starch, haemostasis, kidney.

INTRODUCTION

Many critically ill equine patients require plasma volume expansion. Both crystalloid and colloid solutions can be used for this purpose. Crystalloids rapidly pass across capillary membranes into the extra-vascular space, while colloids are retained in the intra-vascular space for longer. Most colloids are polydisperse solutions [1]. Colloids with low weight-average molecular weight (MWw) are thought to pass through capillary pores at a faster rate than those with higher MWw and are therefore considered to be less efficient plasma volume expanders [2]. However, comparisons of a 4% modified fluid gelatin (MFG) (Gelofusine®)[a] and 6% hydroxyethyl starch (HES) (130/0.4) (Voluven®)[b] in healthy human volunteers did not detect a difference in volume expansion, despite the 100 kDa difference in MWw [3].

In addition to being hypovolaemic, many critically ill horses are hypoproteinaemic, becoming more so following protein dilution subsequent to resuscitation fluid administration [4]. Inadequate serum protein levels can lead to inadequate serum colloid osmotic pressure (COP). Sufficient COP is required to retain fluid within the microvasculature, maintain normovolaemia and prevent oedema formation. Semi-synthetic colloids have been shown to augment COP [5, 6]. This offers the potential to reduce the risk or extent of oedema formation in hypoproteinaemic patients [7].

While a number of studies have described the effects of HES in normal and diseased horses, there is a paucity of information regarding effects of MFGs, despite their reported benefits in other species [8, 9].

Following the recent temporary market withdrawal of HESs in some countries, awareness of the possible deleterious effects of colloids has increased [10]. Concerns associated with semi-synthetic colloid use include renal failure, impaired haemostasis and anaphylactoid reactions. None of these effects have been reported in horses.

This study aimed to compare the effects of a 4% MFG (Gelofusine®)[a] and a 6% HES (130/0.4) (Voluven®)[b] on haemodilution and COP in healthy ponies. In addition, it aimed to determine if either

HES or MFG had any deleterious effects on haemostasis or renal function in healthy ponies at clinically relevant dosages.

LITERATURE REVIEW

Colloid solutions

Introduction to hydroxyethyl starches

HESs are derived from amylopectin and are subsequently hydroxylated to reduce the rate of *in vivo* hydrolysis [1]. HESs are described according to their concentration, relative molecular mass, rate of substitution (hydroxyethylation) and ratio of C2: C6 substitution [1]. For example 6% hydroxyethyl starch (130/0.4) (Voluven®) has a concentration of 60 mg/ml, a relative molecular mass of 130 kDa and a hydroxyethyl substitution rate of 4/10. The C2: C6 substitution ratio is not routinely included in the written nomenclature. The prefixes tetra, penta and heta can be used to describe the hydroxyethylation rates of hydroxyethyl starches, with 40%, 50% and 70% of glucose units hydroxylated respectively.

The greater the number of molecules present within the solution the larger the osmotic pull effected. Higher molecular weight starches are retained within the vasculature for longer but cause less of an osmotic pull. Less of an osmotic pull is achieved due to the fact that fewer molecules are able to fit within any given volume of fluid. A higher rate of substitution and a higher ratio of C2: C6 leads to longer persistence within the vasculature [1].

Introduction to modified fluid gelatins

MFGs are derived from naturally occurring bovine collagen. They are subsequently succinylated in order to induce a conformational change. This change increases the molecular size, without increasing the molecular weight. This enables increased vascular persistence without decreasing the size of the osmotic pull achieved [1]. Modified fluid gelatins are routinely described only by their concentration and molecular weight, but molecular weight is not included in the written nomenclature. For example a 4% MFG (Gelofusine®) has a concentration of 40 mg/ml. The

Beneficial effects of hydroxyethyl starch administration

The augmentation of COP following HES administration was demonstrated in humans [11]. Further to this the effects of colloid solutions on COP in human patients are generally difficult to establish. This is due to the nature of studies reporting their use; large volumes of intravenous crystalloid fluids are almost always concurrently administered [12, 13]. In hypoproteinaemic horses 6% hetastarch causes haemodilution and increased COP [5]. Cardiac index (cardiac output divided by body weight) is also significantly increased following 10% pentastarch infusion in hypovolaemic horses undergoing colic surgery [14]. Most recently significant changes in COP, systolic arterial blood pressure, central venous pressure and packed cell volume were observed following the administration of a 6% tetrastarch to healthy horses [6]. In addition to effects on COP and cardiovascular parameters, HESs also decrease capillary permeability and inflammation. These solutions can decrease capillary leak and trans-endothelial neutrophil migration following hypoxia, blunt trauma and lipopolysaccharide administration [15-17]. The mechanisms involved in these actions are incompletely understood. Vasodilator-stimulated phosphoprotein (VASP) is involved in the control of actin filament geometry [18]. Protein kinase-induced phosphorylation of VASP plays a central role in the maintenance of barrier function [19]. *In vitro* studies have demonstrated that the presence of a protein kinase inhibitor reduces the effects of HES on barrier function, supporting hypotheses that interaction between HESs and VASP may play a role in the mechanism of the effects observed. Whether or not altered interleukin expression plays a role is not confirmed. In septic rats 6% tetrastarch infusion decreases tumour necrosis factor-α, interleukin-1β, macrophage inflammatory protein-2 expression, intercellular adhesion molecule-1 mRNA expression, myeloperoxidase activity and pulmonary neutrophil infiltration compared with saline treated controls [20]. *In vitro* studies have also demonstrated inhibition of neutrophil adhesion and trans-endothelial migration [15].

Beneficial effects of modified fluid gelatin administration

Significant volume expansion has been associated with the administration of a 4% modified fluid gelatin in human critical care patients and dogs [3, 9]. Limited clinical data are available on the use of MFGs for augmentation of COP. Two studies compared the effects of a MFG and a HES on COP in the human ICU patient [12, 13]. Unfortunately in both of these studies the colloid doses were variable and concurrent intravenous crystalloids were also administered. COP was observed to decrease comparably during abdominal aortic aneurysm surgery in patients receiving either HES or MFG [12], but a greater decrease in COP was observed in patients receiving MFG compared with HES during cardiopulmonary bypass [13]. Anecdotal observations within the equine veterinary community show a 4% MFG to affect both plasma volume and COP, although there are currently no published data assessing the efficacy of MFGs in horses. Recently, a 4% modified gelatin was used to achieve acute normovolaemic haemodilution in a horse prior to anticipated intra-operative haemorrhage [21]. The evidence for whether or not MFG administration reduces capillary permeability is mixed. MFG administration reduced capillary permeability in septic rats [20], but in a number of other studies no beneficial effects were observed [22-24].

Adverse effects of hydroxyethyl starch administration

There are a number of concerns associated with the use of HESs, including their effects on renal function. In 2013 a multi-centre review and meta-analysis evaluated the effects of HES administration in human patients. HESs were found to be associated with increased mortality (RR, 1.09; 95% CI, 1.02 to 1.17), increased risk of renal failure (RR, 1.27; 95% CI, 1.09 to 1.47) and increased need for renal replacement therapy (RR, 1.32; 95% CI, 1.15 to 1.50) [25]. It is important to acknowledge that the reviewers identified many of the included papers to have carried a strong bias or confounding factors. This should be considered when contemplating the results of this review. Different HESs have different pharmacokinetic and pharmacodynamic properties [26, 27]. In the 2013 review, no differentiation was made between the effects of different starches on renal function. This may have led to misrepresentation of the effects of newer generation starches. No increase in serum creatinine levels was observed following 6% tetrastarch infusion in horses [28]. These are the only data assessing renal function in horses following the administration of a colloid solution.

In an experimental isolated porcine renal perfusion model, renal interstitial proliferation and tubular damage were observed following infusion with a HES (200/0.5 and 130/0.42) [29]. The exact pathology caused by colloid use is not understood in any species, nor are the underlying pathophysiological processes.

In addition to renal effects there have been concerns pertaining to the effects of HESs on haemostasis. Many *in vitro* studies show clinicopathological alterations following starch infusion, but data demonstrating clinical effects on haemostasis are lacking. A number of papers commonly quoted as demonstrating clinical evidence of hypocoagulability, in fact do not definitively demonstrate this at all, either due to lack of statistical significance or the presence of other relevant non-controlled factors [30-32]. A multicentre review and meta-analysis found no increased risk of bleeding following HES administration [25].

Specific effects on haemostasis in humans include decreased circulating factor VIII and von Willebrand factor (vWF) concentrations, impairment of platelet function and decreased fibrin clot

stabilization [33, 34]. Following HES administration, passive haemodilutional effects such as decreased fibrinogen and haemoglobin concentrations are observed immediately, whilst vWF only reaches its minimum concentration 1-2 hours following colloid administration [34]. This is indicative of additional mechanisms responsible for the observed decrease beyond the dilutional effect. Reductions in fibrinogen and vWF: vWF antigen ratio have been reported in dogs following MFG administration, although no concurrent alterations in prothrombin time (PT) or activated partial thromboplastin time (aPTT) were observed [9].

Viljoen *et al.*, [28] published work using thromboelastography (TEG) to assess haemostasis in ponies receiving different doses of HES. In this study α-angle and R-time were statistically significantly affected by dose. Despite these observed effects, all TEG variables remained within reference ranges and were considered unlikely to be of clinical relevance.

Adverse effects of modified fluid gelatin administration

There is moderate evidence that MFGs (as well as HESs) increase the risk of renal failure following human cardiac bypass surgery when compared with crystalloid administration alone [35]. There is also evidence that the administration of MFGs increases the risk of acute kidney injury in septic human patients [36]. Further work is required to explore this concern, as the current evidence is not without limitations.

MFGs have been shown to reduce proximal tubular reabsorption of low-molecular weight proteins such as globulins. This can lead to increased levels of these proteins in the urine [37, 38]. This has been demonstrated in the absence of N-acetyl-beta-D-glucoaminidase, a proximal tubular lysosomal enzyme used to screen for proximal tubular cell damage in human patients. These findings were considered consistent with transiently altered proximal tubular function in the absence of tubular damage in people [37]. A marked pseudoproteinuria has also been described following MFG administration and is a direct result of cross-reactivity of the gelatins with urine proteins in certain assays used for protein measurement [39]. There are currently no published data exploring the renal effects of MFGs in horses.

There is no clinical evidence in any species that the administration of a MFG increases the risk of bleeding. *In vitro* studies have demonstrated reduced platelet aggregation as well as reduced vWF levels following the administration of a 4% MFG [40, 41]. An in-vitro thromboelastographic study of blood mixed with a MFG demonstrated a decrease in clot weight and strength compared with controls [42], yet another study in elective orthopaedic patients observed hypercoagulability when compared with baseline values [43]. Furthermore, thromboelastographic evaluation of blood samples from human patients who had received MFG infusions also demonstrated decreased clot strength [44]. The clinical significance of these effects is currently unknown.

Anaphylactoid reactions have been reported in man following the administration of colloids. These vary in severity, from mild cutaneous urticaria, to arterial hypotension and death. Urea-linked gelatins were most commonly associated with these types of reaction, however reactions to HESs and MFGs have also been reported [45, 46]. A prospective study in human patients reported the incidence of anaphylactoid reactions following gelatin and artificial starch administration to be 0.4% and 0.06% respectively [45]. There are no published reports of anaphylactoid reactions in veterinary species.

Evaluation of haemostasis

Prothrombin time

Prothrombin time (PT) reflects the efficiency of the conversion of fibrinogen to fibrin through the extrinsic and common haemostatic pathways. PT can be prolonged in the face of hypofibrinogenaemia or with degradation or consumption of factors VII, V, or X [47]. In human patients diagnosed with disseminated intravascular coagulation (DIC), only approximately 50% have a prolonged PT and it has been suggested that measurement of PT is not a sensitive test for coagulopathy [48]. PT has been used to assess haemostasis in horses. Prolonged PTs are reported in cases of colic in adult horses and sepsis in foals [49-51].

Activated partial thromboplastin time

Activated partial thromoplastin time (aPTT) reflects the efficiency of the intrinsic and common pathways in fibrin formation. aPTT becomes prolonged when factors V, VIII, X, IX, XI, XII or II are sufficiently decreased [47]. This may occur through plasmin degradation of these factors. The degree of degradation of coagulation factors depends on the degree of activation of the fibrinolytic system in response to a triggering event. Similar to PT, hypercoagulability or circulating fibrinogen degradation products can affect aPTT. This is a concern when using aPTT as a test for coagulopathy [48].

Like PT, prolonged aPTT has been described in horses with acute abdominal disease and in foals with septicaemia [49-51].

Thromboelastography

TEG is a viscoelastic, whole blood based assay that analyses both the cellular and soluble components of the coagulation process. Compared with other methods of assessing coagulation it provides a more global assessment of the haemostatic system. It detects changes in the viscoelastic properties of a blood clot, from its formation through to fibrinolysis [52]. Thus, in essence, TEG measures the ability of a clot to perform mechanical work throughout its structural development. A significant advantage of TEG over conventional standard plasma-based assays is that it allows for identification of either hypo- or hypercoagulability in whole blood samples. It assesses whole blood coagulation rather than individual components of the coagulation pathway. Three slightly different techniques can be used to perform TEG assays: Whole citrated blood, citrated kaolin-activated or citrated tissue factor-activated blood can be used.

The TEG variables commonly measured in horses include the following:

Reaction time (R-time): This is the period of time elapsing from when blood is placed in the TEG analyser until detectable clot formation occurs. It is correlated with the activity of plasma coagulation factors. Reaction time can be shortened by hypercoagulable conditions such as DIC and prolonged by factor deficiencies and anticoagulant agents.

K-time: This represents a measure of the speed of clot formation and is typically characterised as the time from the end of R measurement until a defined clot strength is achieved. K-time is related to clot kinetics and corresponds to the initial activation of platelets and fibrinogen. It can be shortened by increased fibrinogen concentrations and prolonged by anticoagulants and hypofibrinogenaemia.

Alpha angle (α-angle): This is closely related to K-time and corresponds to the slope of the tangent on the elasticity curve and indicates a tendency towards hypo- or hypercoagulable conditions. It measures the rapidity of clot strengthening.

Maximum amplitude (MA): This represents the overall clot strength and depends on the contribution of two components: primarily platelet aggregation and a modest contribution of fibrin to clot strength.

G-value: This is calculated using the MA and represents the viscoelastic strength of the clot.

LY30 and LY60: These provide evidence of thrombolysis. They are measures of amplitude reduction 30 and 60 min after MA respectively and are measured as a percentage decrease in area under the curve at 30 and 60 min compared with MA.

Non-activated TEG has been used to assess coagulability in horses with inflammatory gastrointestinal disorders that were considered likely to have a coagulopathy [53, 54]. Although this technique identified a tendency towards increased coagulability in the affected versus healthy horses, there was a significant overlap in values between the two groups. Non-activated TEG has also been used to assess haemostasis in septic foals and cases of exercise-induced pulmonary haemorrhage [53, 55].

In light of the variability of results observed from non-activated TEG assays in horses, activated assays were investigated for use in the horse [56]. These authors found significant differences between non-activated and activated assays for all measured parameters (P <.05). The intra-individual variation was significantly higher with the non-activated assay compared with tissue-factor and kaolin-activated protocols. Additionally, the inter-individual variation was lowest for kaolin-activated TEG assays,

leading to the recommendation of this method of activation when performing TEG assays in horses. Recently, when kaolin-activated TEG was used to assess haemostasis in ponies receiving different doses of an HES, α-angle and R-time were significantly affected by dose [28].

Urinalysis

Urine gamma glutamyltransferase: creatinine ratio

Urinary gamma glutamyltransferase (GGT) may be expressed as a ratio to urinary creatinine (UGC). Creatinine is neither actively excreted nor absorbed. This means that when enzyme levels are considered in a ratio with creatinine, they become adjusted for urine concentration. The measurement of urinary levels of renal tubular enzymes is an established test in the assessment of renal tubular damage in human patients. Unfortunately there are significantly less data available on the use of urinary enzymology in the assessment of the equine renal patient. Constitutive renal tubular enzymes are present in intracellular lysosomes and at the brush border. Depending on the assay, low levels are usually detectable in the urine, as renal tubular cell turnover is a physiological process [57]. During periods of inflammation or necrosis of the renal tubules urine enzyme levels may be increased. GGT is the most studied enzyme in equine nephrology, although data are still limited. GGT is a brush border enzyme of the proximal tubule. Hinchcliff *et al.,* found increased UGC in ponies receiving gentamicin compared with baseline values [58]. Furthermore, these changes preceded the development of azotaemia, indicating that UGC may be useful as an early indicator of renal damage, potentially enabling the identification of patients with renal disease prior to the loss of 75% of nephrons. Interestingly, UGC increased quickly following gentamicin administration. These authors described normal UGC to be <25 IU/g. During gentamicin administration but prior to azotaemia, UGC of 25-100 IU/g were observed and following the onset of azotaemia values greater than 100 IU/g were observed. The clinical relevance of UGC ratios between 25 and 100 IU/g remains debatable. Monitoring a trend in an individual patient may enable a rational decision to be made regarding the benefits of the drug versus the increasing level of proximal tubular damage. It should also be considered that GGT is produced by other organs of the body, in particular the liver. Circulating levels of this enzyme should not affect urinary GGT levels as this enzyme is not normally filtered by the glomerulus. Although

secretion of GGT is specific to proximal tubular cells, urinary levels may also be increased in the presence of glomerular damage due to subsequent filtration of this molecule. In the presence of a concurrent proteinuria this should be considered.

Urine protein: creatinine ratio

Urine protein: creatinine ratios (UPCs) are more commonly used than total urine protein measurements. This allows the effect of urine concentration on the measured protein level to be eliminated. Protein in the urine may be of pre-renal, renal or post-renal origin. Pre-renal refers to the presence of abnormal amounts of protein in the plasma and can include molecules such as haemoglobin, myoglobin and Bence-Jones proteins (immunoglobulin light chains) [59]. These are not indicative of renal disease but may, depending on the protein, be liable to cause renal injury. Renal proteinuria refers to increased proteins in the urine due to abnormal renal handling of normal amounts of plasma proteins. This can be due to structural or functional lesions. Renal proteinuria is most commonly associated with increased filtration of plasma proteins due to glomerular disease. It can however, also occur with proximal tubular disease; low molecular weight proteins can be filtered by the normal glomerulus and are subsequently reabsorbed in the proximal tubule [60]. Defects in this process can lead to increased amounts of low molecular weight proteins (and small amounts of albumin) in the urine. Interstitial renal disease i.e. infection or inflammation of the interstitium, can lead to increased urinary proteins subsequent to secretion of proteins into the peri-tubular capillaries and then into the renal tubules. Post-renal proteinuria refers to the presence of proteins secreted into the urine distal to the kidney. This is usually associated with inflammation or infection of the urinary tract or genitalia and can also be seen subsequent to haemorrhage in these regions [59]. Pre-renal proteinuria can be identified by increased levels of plasma proteins upon biochemical assay. If increased, protein electrophoresis may be useful in identifying the types of proteins responsible. The interpretation of renal versus post-renal protein increases is not always simple. Non-laboratory based diagnostics such

as cystoscopy and transabdominal ultrasonography may help to identify possible causes of either renal of post- renal protein sources. Urine may be collected from each ureter individually under cystoscopic guidance. Protein assays on these samples should enable differentiation between proteinuria originating from the kidney or ureter, versus the bladder, urethra and genitalia.

STUDY OBJECTIVE

1. To determine the effects of two alternative 4% MFG dosage regimens and a 6% HES (130/0.4) regimen on haemodilution, COP, haemostasis and renal parameters in healthy ponies.

STUDY HYPOTHESES

1. The administration of either a 4% MFG or a 6% HES (130/0.4) to healthy ponies at a bolus dose of 10 ml/kg will lead to a significant increase in haemodilution and COP when compared with baseline values.

2. The size of the effects of a 4% MFG on haemodilution and COP will be greater following a 20 ml/kg dose than a 10 ml/kg dose.

3. 4% MFG and a 6% HES (130/0.4) administration will not have a significant effect upon haemostatic or renal parameters.

MATERIALS AND METHODS

Animals

Six healthy, adult non-pregnant female Nooitgedacht ponies belonging to the University of Pretoria were selected for the study. The ponies had a mean age of 10.5 years old (range 8-15 years) and a mean body weight of 408 kg (range 377-428 kg). Ponies were determined to be clinically healthy on the basis of normal clinical examination, complete blood count (CBC), serum biochemistry, coagulation profile (including TEG), urinalysis and abdominal ultrasonography prior to enrolment.

Fluid administration

A randomised crossover design was used with a one week washout period between each treatment. The 6 ponies were randomly assigned to treatment ordering using a Latin Square design. All ponies received each treatment as described below. Treatment A = HES (Voluven®)[b] bolus of 10 ml/kg administered intravenously over a constant time period (approximately 20 min). Treatment B = MFG (Gelofusine®)[a] bolus of 10 ml/kg administered intravenously over constant time period (approximately 20 min). Treatment C = MFG (Gelofusine®)[a] bolus of 20 ml/kg intravenously over a constant time period (approximately 40 min). Ponies were stabled approximately 18 h prior to fluid administration and an intravenous 12G catheter (Equivet HiFlow Long Term IV Catheter 12G x 5.25")[c] was aseptically placed in the jugular vein at this time. Fluids were administered by gravity through an administration set (Y Type Hi-Flo Blood Admin Set)[d]. Fluid bags were suspended at a height of 2.5 metres above the ground (Fig 1). Intravenous catheters were removed after the sampling period and replaced immediately prior to the next sampling period, alternating between the left and the right jugular.

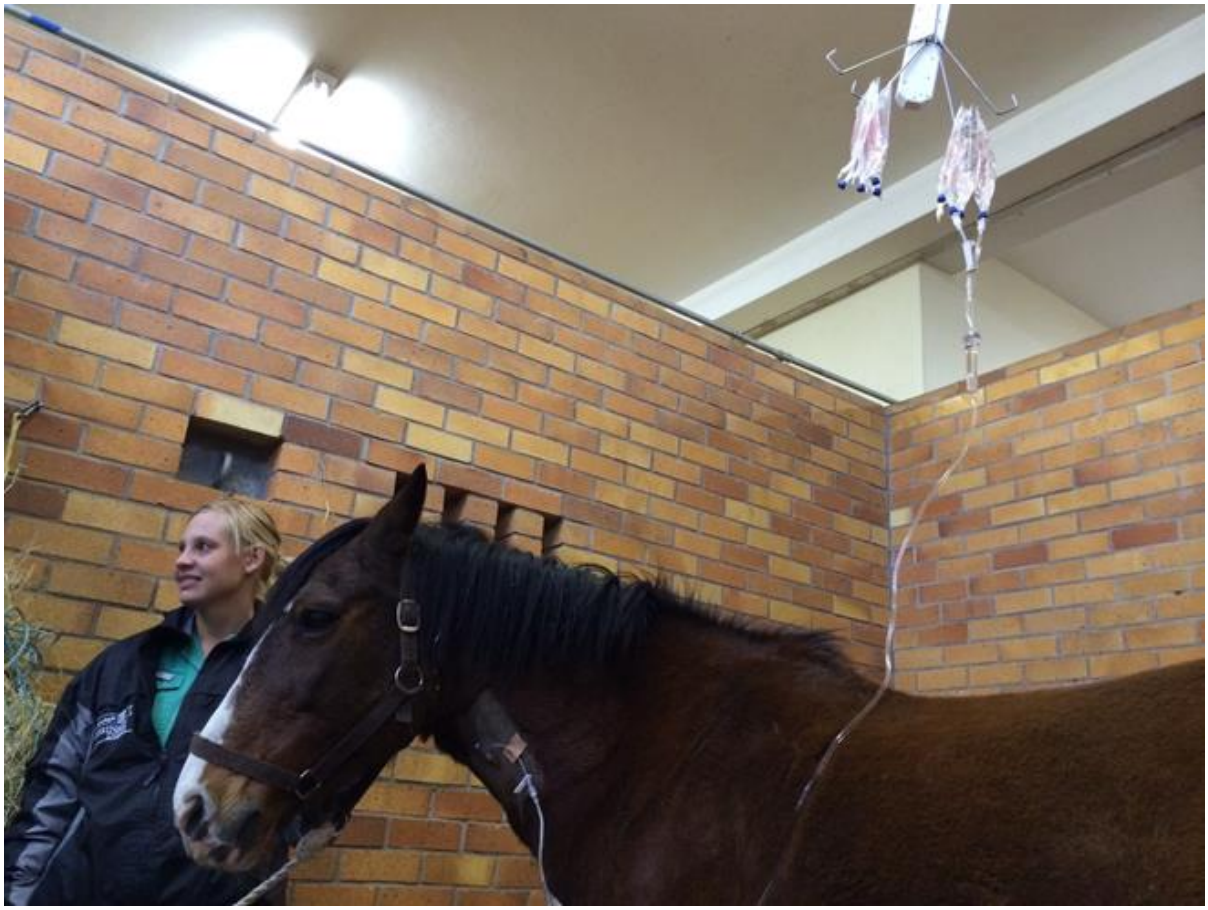

Figure 1. Treatment administration

During the treatment and sampling periods the ponies were kept stabled and received *ad libitum* access to water and grass hay (*Eragrostis tef)*. Ponies were returned to pasture between treatments and after completion of the study.

Sampling

Haematocrit, total serum protein (TSP) and COP were measured at baseline (1 h prior to infusion), time 0 (immediately after infusion completed) and at 1, 2, 3, 6, 12 and 24 h after time 0. Platelet count, aPTT, PT, plasma fibrinogen concentration and TEG variables (measurements of reaction time (R), coagulation time (K and α-angle), clot strength (MA and G value) and clot lysis (LY30 and LY60), were measured at baseline and at 1, 6, 12 and 24 h after time 0. Serum creatinine concentration, urine protein: creatinine ratio (UPC), urine gamma glutamyltransferase: creatinine ratio (UGC) urine specific gravity (USG) and urine sediment examination (USE) were measured at stabling and at 24 h after time 0. These renal parameters were also measured one week after the final treatment administration.

Blood samples were obtained from the i.v. catheter (Fig 2). On each occasion the catheter was flushed with 10 ml sterile non-heparinised saline, 20 ml of blood was collected and discarded, the sample (20 ml) was collected and the catheter was flushed again with 10 ml sterile non-heparinised saline. Samples for creatinine, TSP and COP testing were transferred into serum tubes containing a clot activator[e]. Samples for CBC, haematocrit and platelet count were transferred into EDTA tubes[e]. Samples for TEG, aPTT, PT and fibrinogen concentration were transferred into citrate tubes[e] containing 3.2% citrate for a final citrate: blood ratio of 1: 9. Urine samples were collected via aseptic urinary bladder catheterisation.

A clinical examination was performed prior to each infusion, and subsequently at each sampling point.

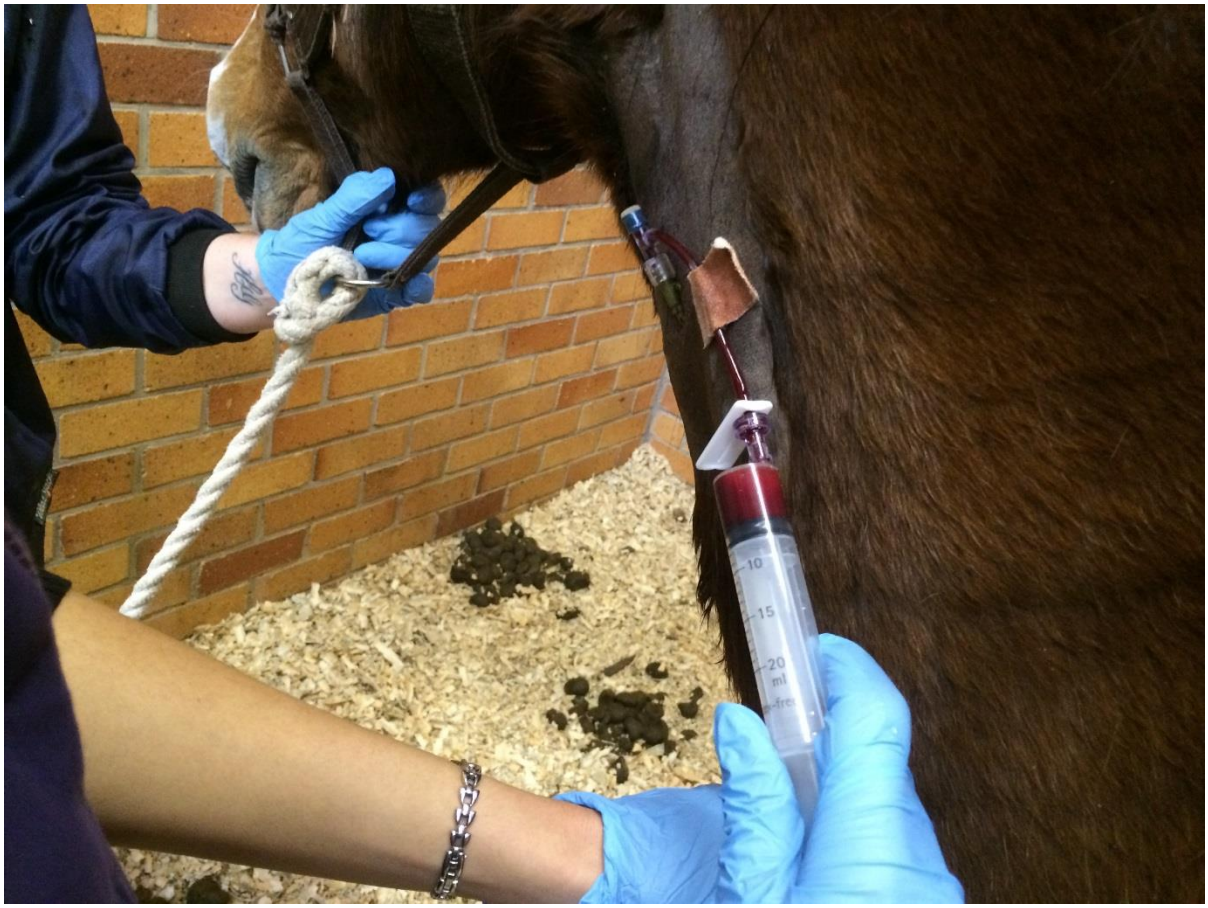

Figure 2. Sample collection

Sample processing and analysis

Blood for serum samples was centrifuged (Hettich Universal 320 Centrifuge)[f] at 2,100 *g* for 8 min, separated and frozen at -20ºC until testing. Serum samples were subsequently thawed and processed as a batch with creatinine and TSP measured using an automated serum biochemistry analyser (Cobas Integra 400 plus)[g] and serum COP measured using a serum colloid osmometer (Wescor Model 4420)[h]. Complete blood count, haematocrit and platelet count values were determined using an automated analyser (Advia 2120 Hematology System)[i]. Separate citrate tubes were collected for TEG versus aPTT, PT and fibrinogen. TEG analyses were performed 30 min after collection using a thromboelastograph machine (Thromboelastograph Hemostasis System 5000)[j] and a standardised protocol: Thromboelastograph balance and e-test (which measures the zero point) were verified prior

to each analysis. The citrated blood samples were maintained at room temperature for 30 min prior to analysis. Twenty µL of 0.2M calcium chloride was added to a new preheated (37˚C) reaction cup in one of the TEG channels. The blood in the citrated tube was mixed by inverting the tube 5 times prior to adding 1 ml of blood to the tube containing kaolin. The kaolin tube with added blood was then inverted 5 times enabling mixing of the kaolin and citrated whole blood. Next, 340 µL kaolin-activated citrated whole blood was added to each reaction cup and samples were run. The second tube of citrated blood was centrifuged (Hettich Universal 320 Centrifuge)[f] at 2,100 *g* for 8 min, separated and frozen at -80˚C. Citrated plasma was subsequently thawed and aPTT, PT and fibrinogen assays performed as a batch.

For USE, the urine was centrifuged (Hettich Universal 320 Centrifuge)[f] at 790 *g* for 10 min and the sediment re-suspended using Sedi-stain[k]. Sediment smears were then manually prepared and viewed immediately. Urine supernatant was frozen and stored at -80˚C until analysis. Samples were subsequently processed as a batch, with GGT, protein and creatinine values being obtained using an automated biochemistry machine (Cobas Integra 400 plus)[g]. UPC and UGC were calculated manually.

Data analysis

Data were assessed for normality by evaluating descriptive statistics, plotting histograms and performing the Anderson-Darling normality test within statistical software (Minitab Statistical Software, Release 13.32)[l]. Data were descriptively presented as median and ranges due to the small sample sizes per time period and the apparent violation of the normality assumption for a number of analysed outcomes. Data that were not normally distributed were transformed using either the natural logarithm or by ranking prior to statistical analysis. A general linear modelling approach was used that incorporated adjustment for the repeated sampling of horses. Linear mixed models including fixed effect terms for treatment group, time of sampling, week of the study and an interaction between treatment and sampling time. A random effect term was included for horse and a first order

autoregressive correlation structure was used to adjust for repeated measurements. *Post- hoc* comparisons were adjusted using Bonferroni correction of *P* values. Renal outcomes were compared over time and treatment using Friedman tests and pre- and post-study using Wilcoxon signed-rank tests. Statistical analyses were performed in commercially available software (Minitab Statistical Software, Release 13.32 and IBM SPSS Statistics Version 22)[lm] and results interpreted at the 5% level of significance.

RESULTS

Clinical parameters remained within normal limits for all ponies throughout the study. No anaphylactoid reactions were recorded. Mild phlebitis occurred in 7/18 (39%) treatment episodes without an apparent association between treatment type or study week. Ultrasonography of the affected veins confirmed phlebitis without the presence of visible thrombi. In these cases the i.v. catheters were removed and samples subsequently collected via venepuncture. Phlebitis was treated with topical application of ice and a combination of dimethyl sulfoxide and dexamethasone gel (Dexamethasone/DMSO Gel)[n].

Haemodilution

Haematocrit decreased significantly from baseline following all three treatments (Fig 3; Table 1; *P*<0.001). Following treatments A and B haematocrit was significantly decreased until 6 h post infusion, whilst following treatment C it remained significantly decreased until 12 h post-infusion. There was no significantly different effect on haematocrit between treatments A and B, but treatment C caused a significantly larger decrease in haematocrit than both other treatments.

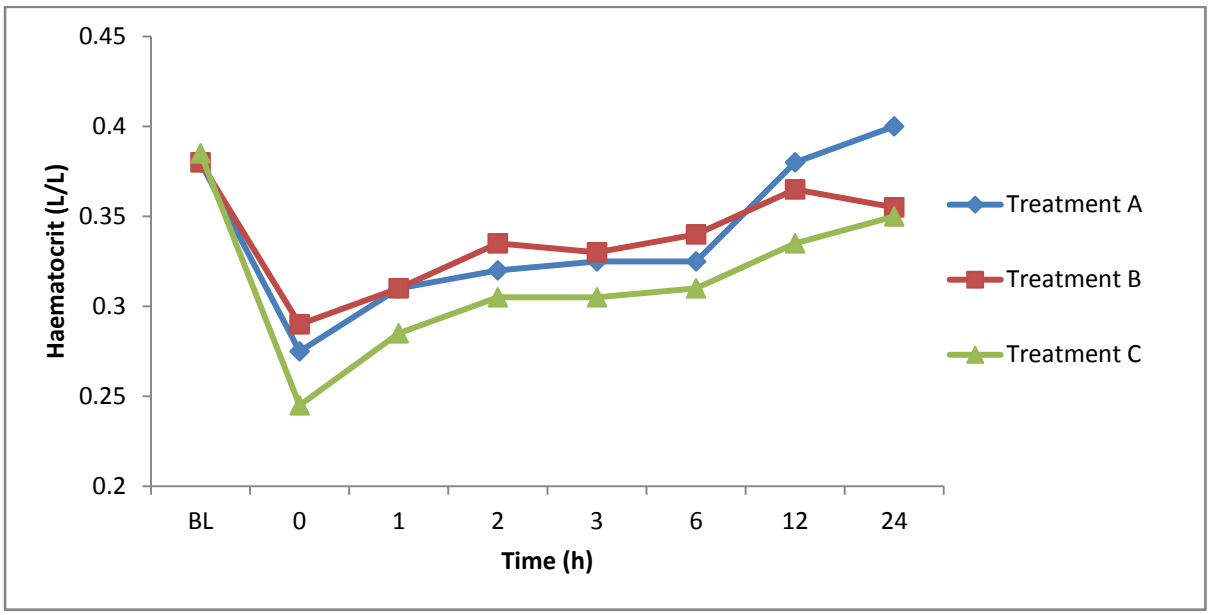

Figure 3. Effect, median, of each of the 3 treatments on haematocrit over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt. BL= baseline.

No significant changes in platelet count were seen following the administration of treatments A and B, although the median platelet count was lower than baseline for all time points following both treatments (Fig 4; Table 1; $P = 0.08$ and $P = 0.5$ respectively). Platelet counts following treatment C changed over time ($P<0.001$) and were significantly lower than baseline for 24 h. Treatment C had a significantly greater effect on platelet count than treatment B.

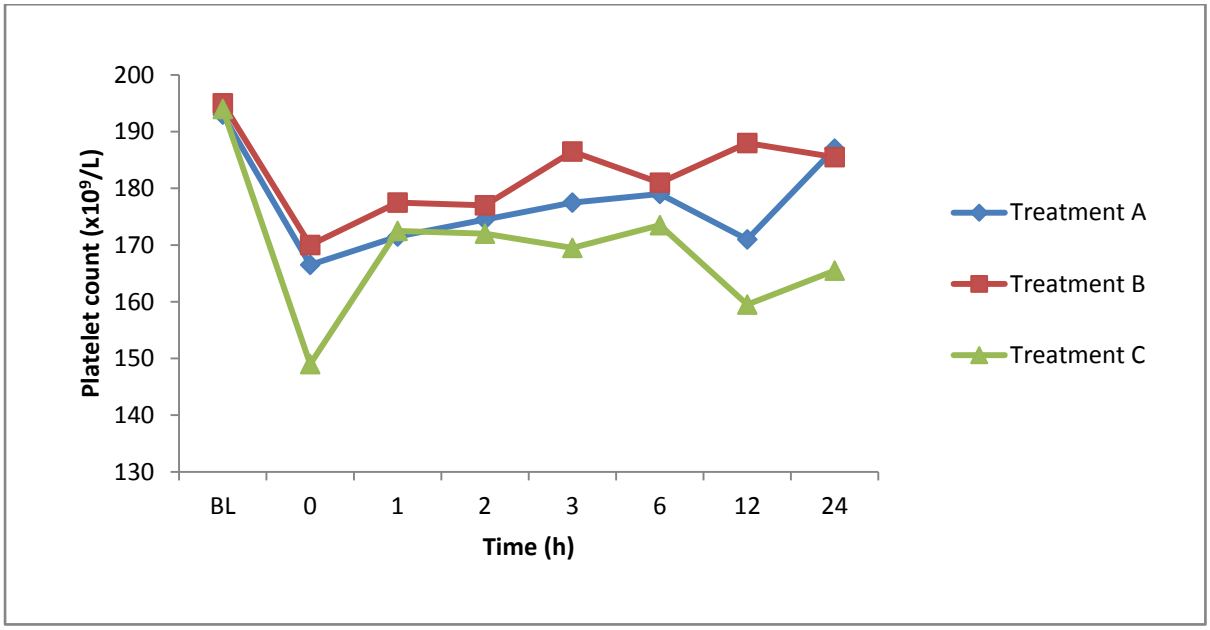

Figure 4. Effect, median, of each of the 3 treatments on platelet count over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt. BL= baseline.

TSP was significantly reduced from baseline following all three treatments (Fig 5; Table 1; $P<0.001$) and treatment A had a significantly greater effect than treatment B. There was no significant difference in TSP between either treatments A and C, or treatments B and C.

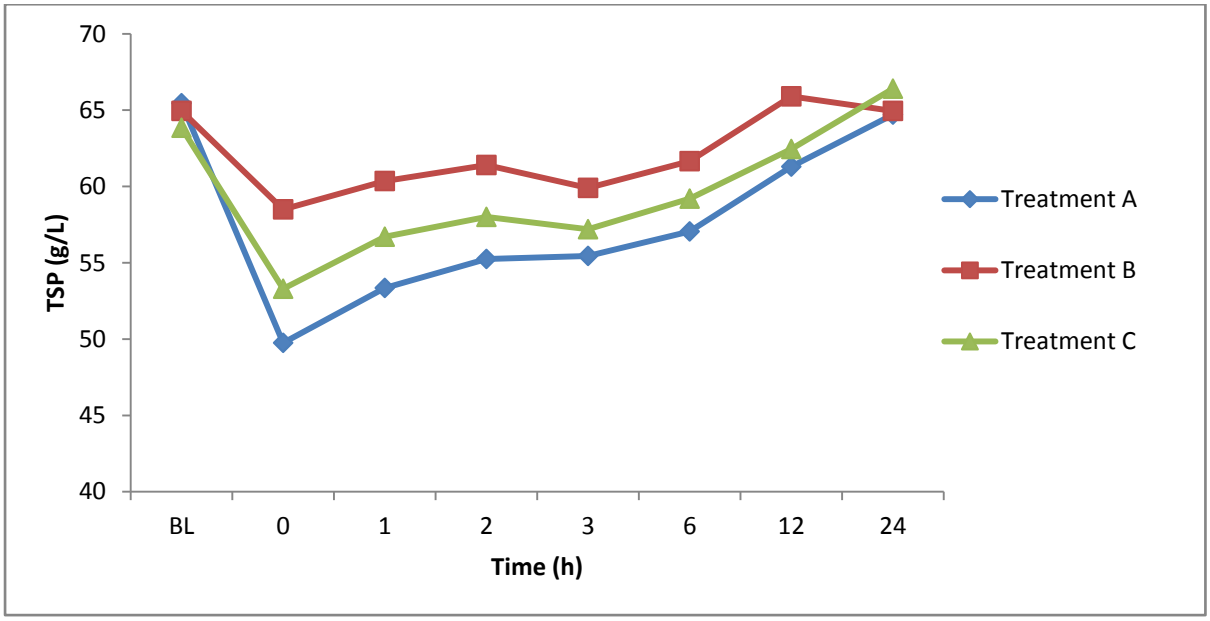

Figure 5. Effect, median, of each of the 3 treatments on TSP over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt. BL= baseline.

Table 1. Haemodilutional variables, median (range), after infusion of each of the 3 treatments. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt.

Variable	Treatment	Baseline	0 h	1 h	2 h	3 h	6 h	12 h	24 h	*P* value*
Haematocrit (L/L)	**A**[1]	0.38 (0.35-0.46)	0.26* (0.27-0.31)	0.31* (0.29-0.33)	0.32* (0.31-0.35)	0.33* (0.3-0.35)	0.33* (0.31-0.35)	0.38 (0.31-0.40)	0.40 (0.35-0.44)	<0.001
	B[1]	0.38 (0.33-0.43)	0.29* (0.26-0.31)	0.31* (0.30-0.34)	0.34* (0.31-0.36)	0.33* (0.31-0.35)	0.34* (0.32-0.38)	0.37 (0.30-0.39)	0.36 (0.33-0.39)	<0.001
	C[2]	0.39 (0.34-0.43)	0.25* (0.24-0.26)	0.29* (0.23-0.33)	0.31* (0.28-0.34)	0.31* (0.26-0.32)	0.31* (0.29-0.38)	0.34* (0.29-0.37)	0.35 (0.32-0.41)	<0.001
										0.001†
Platelet count (x 10^9/L)	**A**[1,2]	193.0 (121-237)	166.5 (127-207)	171.5 (140-218)	174.5 (116-217)	177.5 (110-217)	179.0 (151-225)	171.0 (133-221)	187.0 (130-242)	0.08
	B[2]	195.0 (122-240)	170.0 (141-201)	177.5 (123-230)	177.0 (131-231)	186.5 (143-216)	181.0 (147-232)	188.0 (140-237)	185.5 (137-235)	0.5
	C[1]	194.0 (141-228)	149.0* (124-168)	172.5* (135-187)	172.0* (128-190)	169.5* (141-183)	173.5* (141-201)	159.5* (117-190)	165.5* (141-210)	<0.001
										0.04†
TSP (g/L)	**A**[1]	65.45 (60.8-68.8)	49.75* (48.2-51.4)	53.35* (52.4-55.6)	55.25* (53.4-56.4)	55.45* (53.3-58.1)	57.05* (55-58.7)	61.30* (59.7-64.6)	64.70 (62-68.5)	<0.001
	B[2]	64.95 (60.6-66.6)	58.50* (53.9-60.3)	60.35 (59.2-62.5)	61.40 (58.6-64.2)	59.90* (49.0-63.3)	61.65 (59.4-63.7)	65.90 (61.5-67.6)	64.95 (60.3-70.0)	<0.001
	C[1,2]	63.85 (60.3-71.4)	53.30* (51.4-55.7)	56.70* (53.5-61.2)	58.00* (54.1-62.9)	57.20* (55.8-63.0)	59.20* (55.0-63.9)	62.45 (57.6-64.7)	66.40 (60.6-67.7)	<0.001
										0.03†

***Based on mixed effects linear model with comparisons over time within each treatment group. Medians with asterisks are significantly different than baseline after Bonferroni correction of *P* values for multiple *post- hoc* comparisons. †Effect of treatment over all time points. Treatments without superscripts in common are significantly different after Bonferroni correction of *P* values for multiple *post- hoc* comparisons.**

Colloid osmotic pressure

COP changed significantly over time (Fig 6; Table 2; $P<0.001$) for all three treatments and the effect lasted for 24 h. There was no significant difference between treatments ($P = 0.4$). Treatments B and C had a descriptively greater magnitude short-term increase in COP compared with treatment A.

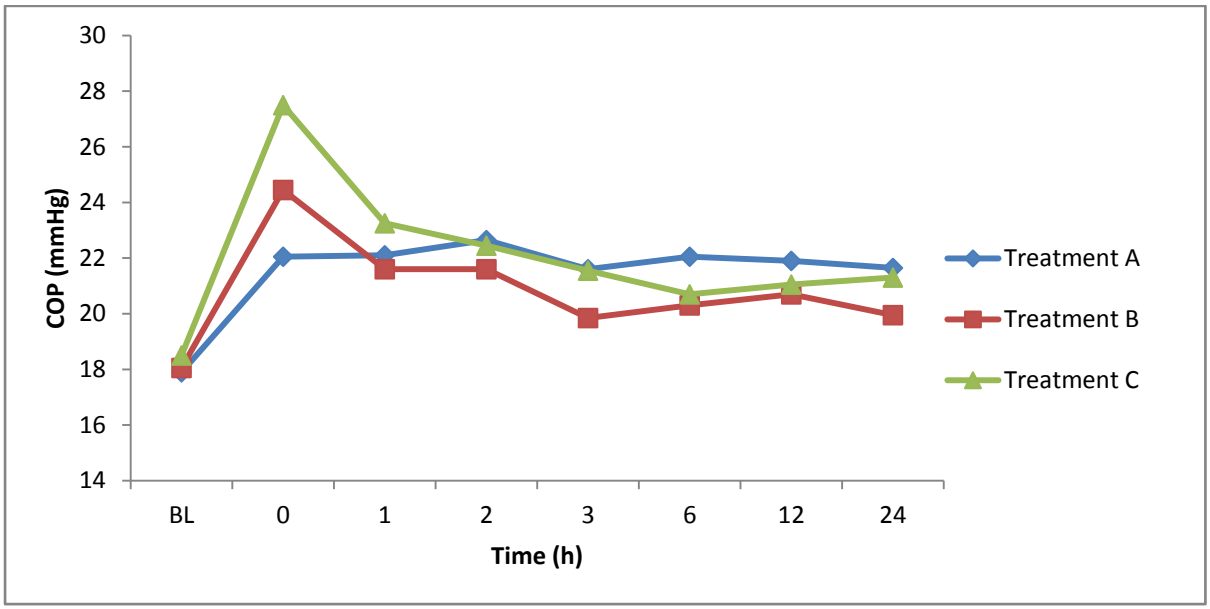

Figure 6. Effect, median, of each of the 3 treatments on COP over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt. BL= baseline.

Table 2. COP, median (range), after infusion of each of the 3 treatments. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt.

Variable	Treatment	Baseline	0 h	1 h	2 h	3 h	6 h	12 h	24 h	*P* value*
COP (mmHg)	**A[1]**	17.90 (16.8-19.5)	22.05* (20.9-23.1)	22.10* (17.3-23.1)	22.65* (19.5-23.9)	21.60* (20.2-22.8)	22.05* (19.9-22.6)	21.90* (20.3-22.8)	21.65* (20.5-24.2)	<0.001
	B[1]	18.05 (14.7-19.4)	24.45* (23.3-26.4)	21.60* (17.1-24.7)	21.60* (20.8-22.3)	19.85 (17.9-21.1)	20.30* (19.0-21.4)	20.70* (18.4-22.2)	19.95* (18.4-22.5)	<0.001
	C[1]	18.50 (16.7-20.1)	27.50* (25.5-30.0)	23.25* (19.2-25.4)	22.45* (20.4-24.3)	21.55* (19.7-24.0)	20.70* (18.8-23.0)	21.05* (18.6-21.9)	21.30* (17.9-22.9)	<0.001
										0.4†

***Based on mixed effects linear model with comparisons over time within each treatment group. Medians with asterisks are significantly different than baseline after Bonferroni correction of *P* values for multiple *post- hoc* comparisons. †Effect of treatment over all time points. Treatments without superscripts in common are significantly different after Bonferroni correction of *P* values for multiple *post- hoc* comparisons.**

Haemostasis

There were no significant effects of treatment or time on R, K, α-angle, MA or G. However, LY30 varied over time after treatment A (Table 3; $P = 0.04$) with no apparent relationship to baseline and LY60 had a significant overall treatment effect ($P = 0.05$), but with no significant pairwise differences after adjusting P values for multiple *post- hoc* testing.

PT changed significantly over time following treatments A and C (Fig 7; Table 3; $P = 0.001$, $P = 0.01$ respectively), but no significant changes were observed following treatment B ($P = 0.06$). The effect on PT was not significantly different between treatment groups ($P = 0.3$).

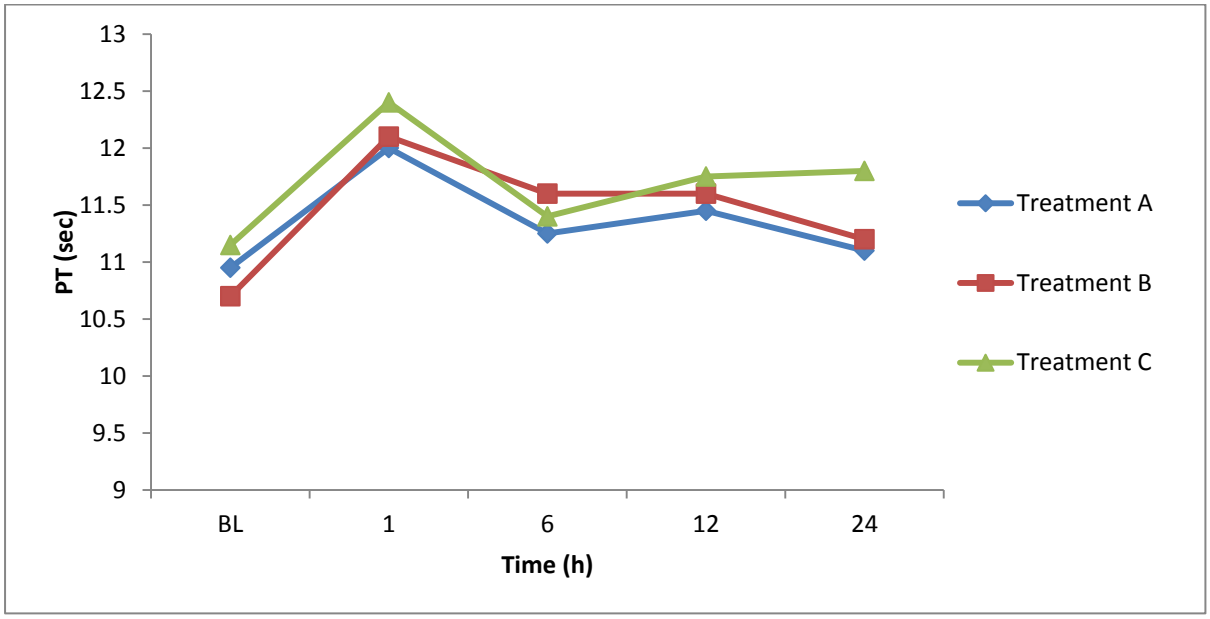

Figure 7. Effect, median, of each of the 3 treatments on PT over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt. BL= baseline.

aPTT changed significantly over time following treatments A and B (Fig 8; Table 3; $P = 0.002$, $P = 0.03$ respectively), but no differences were observed following treatment C ($P = 0.2$). The effect on aPTT was not significantly different between treatment groups ($P = 0.5$).

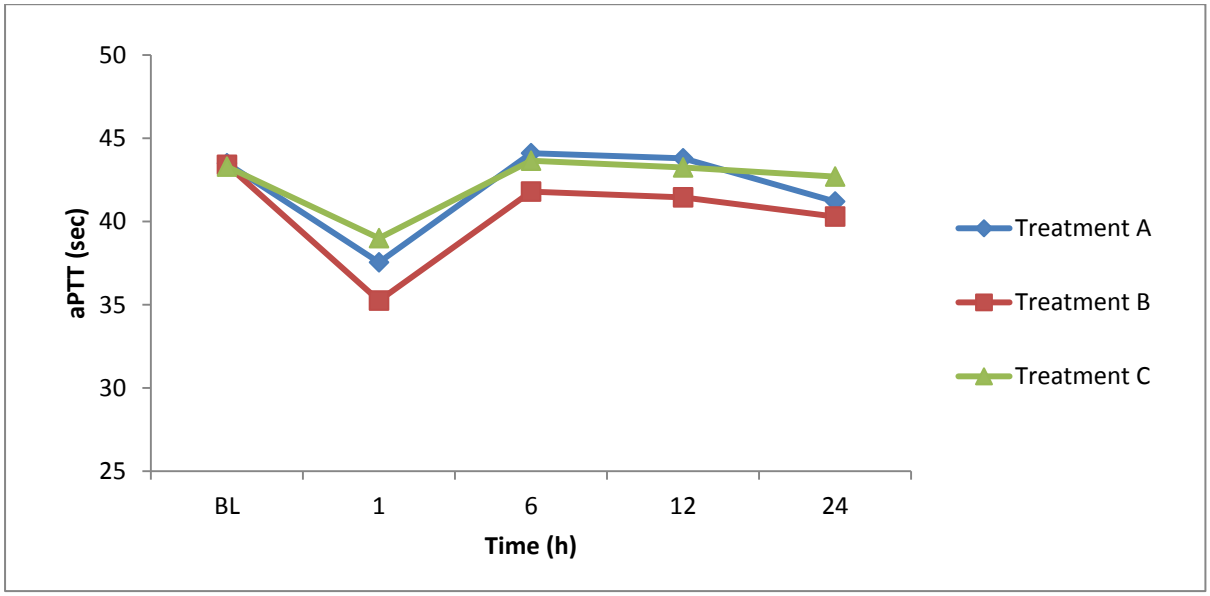

Figure 8. Effect, median, of each of the 3 treatments on aPTT over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt. BL= baseline.

Fibrinogen concentration changed significantly over time following treatments A and C (Fig 9; Table 3; $P = 0.003$, $P<0.001$). After administration of treatment C, fibrinogen concentration was significantly lower than baseline until 6 h post-infusion and then increased to greater than baseline at 24 h. The effect on fibrinogen concentration was not significantly different between treatment groups ($P = 0.1$).

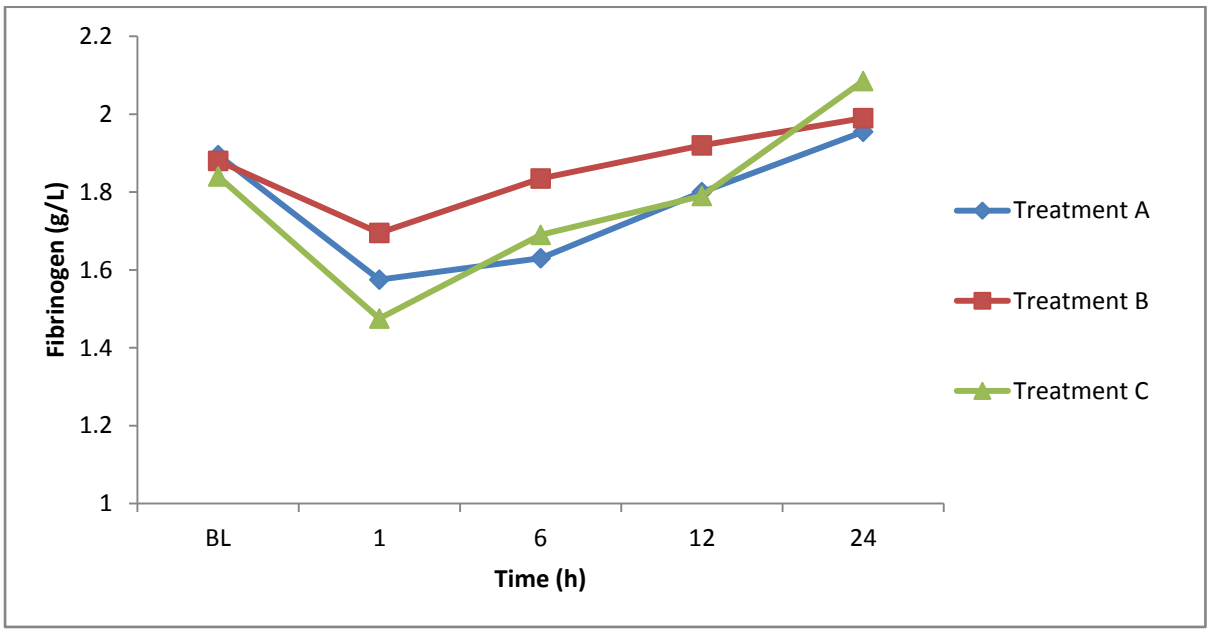

Figure 9. Effect, median, of each of the 3 treatments on fibrinogen over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt. BL= baseline.

Table 3. Haemostatic variables, median (range), after infusion of each of the 3 treatments. Treatment A = HES (Voluven®) bolus of 10 ml/kg. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg.

Variable	Treatment	Baseline	1 hr	6 hr	12 hr	24 hr	*P* value *
R (min)	**A**[1]	17.60 (14.5-27.8)	14.90 (8.5- 17.0)	16.90 (11.2-19.2)	15.65 (9.2-18.4)	15.00 (8.9-21.1)	0.06
	B[1]	17.55 (12.8-22.5)	14.20 (10.8-19.8)	14.10 (10.6-26.3)	13.30 (10.9-21.1)	14.00 (9.5-22.1)	0.3
	C[1]	17.20 (15.2-21.6)	16.30 (11.5-18.6)	16.15 (12.8-19.2)	17.75 (8.5-24.4)	15.20 (7.9-18.6)	0.3 0.2†
K (min)	**A**[1]	4.90 (4.0-7.3)	4.15 (3.5-5.3)	3.80 (2.5-5.8)	3.90 (3.0-4.6)	4.10 (2.6-7.0)	0.1
	B[1]	5.55 (3.2-7.8)	4.05 (2.8-8.3)	3.80 (2.8-5.3)	3.35 (3.2-5)	3.90 (2.9-4.3)	0.1
	C[1]	4.55 (3.9-7.2)	4.00 (3.1-4.5)	3.90 (2.8-4.7)	4.15 (3.3-6)	3.70 (2.7-5)	0.06 0.2†
α (°)	**A**[1]	37.70 (32.8-44.2)	42.20 (25.3-46.8)	47.35 (33.9-57.7)	46.10 (39.6-52.6)	43.60 (33-54.3)	0.1
	B[1]	39.35 (23.4-51.8)	41.55 (26.2-54.3)	42.45 (39.7-57.3)	46.25 (35.7-50.7)	44.55 (42.4-54 4)	0.3
	C[1]	41.40 (28.6-45.8)	43.80 (25.9-50.4)	44.25 (33.2-53.8)	38.95 (34.3-48.6)	47.70 (43.3-54.9)	0.2 0.5†
MA (mm)	**A**[1]	57.20 (49.5-71.1)	57.40 (51.7- 67.2)	57.50 (53.3-69.0)	59.60 (53.2-64.3)	54.75 (48.5-72.9)	0.9
	B[1]	59.30 (44.5-69.5)	62.05 (47.3-69.8)	64.4 (43.9-72)	62.35 (52.1-67.6)	60.85 (50.0-69.8)	0.5
	C[1]	59.00 (48.6-72.9)	65.70 (53.6-68.2)	65.20 (49.9-71.8)	60.65 (37.5-68.8)	63.55 (46.6-72.6)	0.3 0.1†

Table 3 continued. Haemostatic variables, median (range), after infusion of each of the 3 treatments.

Treatment A = HES (Voluven®) bolus of 10 ml/kg. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg.

Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg.

Variable	Treatment	Baseline	1 hr	6 hr	12 hr	24 hr	*P* value *
G (d/s)	**A**[1]	6.70	6.75	6.75	7.35	6.05	0.9
		(4.9-12.3)	(5.3-10.2)	(5.7-11.1)	(5.7-9)	(4.7-13.4)	
	B[1]	7.30	8.30	9.45	8.35	7.80	0.6
		(4-11.4)	(4.5-11.5)	(3.9-12.9)	(5.4-10.5)	(5-11.5)	
	C[1]	7.25	9.55	9.45	7.75	8.7	0.08
		(4.7-13.5)	(5.8-10.7)	(5-12.8)	(3-11)	(4.4-13.3)	
							0.6†
LY30 (%)	**A**[1]	0.75	0.45	1.00	0.65	1.5	0.04
		(0.0-3.0)	(0.0, 2.9)	(0.4-3.9)	(0.2-4.2)	(0.2-4.1)	
	B[1]	0.45	0.35	1.05	0.60	0.45	0.3
		(0.1-2.1)	(0-1.6)	(0-2.8)	(0.1-3.8)	(0.1-2.2)	
	C[1]	0.15	0.25	0.35	0.80	0.25	0.2
		(0.0-3.1)	(0.0-3.5)	(0.0-3.5)	(0.1-7.6)	(0.0-2.9)	
							0.06†
LY60 (%)	**A**[1]	4.00	4.30	5.00	4.30	6.10	0.08
		(2.6-10)	(3.1-9.5)	(4.1-11.4)	(3.1-10.7)	(3.2-11.4)	
	B[1]	3.80	3.40	5.15	4.00	3.90	0.4
		(2.5-7.9)	(1.7-6.9)	(2.3-9.8)	(2.5-10.3)	(1.9-8.5)	
	C[1]	2.95	2.95	3.15	4.60	2.85	0.1
		(1.3-9.7)	(0.6-10.4)	(0.8-9.5)	(2-16.4)	(1.1-9.9)	
							0.05†
PT (s)	**A**[1]	10.95	12.00*	11.25	11.45*	11.10	0.001
		(10.6- 11.6)	(11.8-12.5)	(11-12.5)	(11.2-12.3)	(11- 11.2)	
	B[1]	10.70	12.10	11.60	11.60	11.20	0.06
		(10.5-11.3)	(11.5- 2.4)	(10.9-12.9)	(11.2- 13.5)	(10.7- 13.3)	
	C[1]	11.15	12.40*	11.40	11.75	11.80	0.01
		(10.6-11.6)	(11.7-13.1)	(11.1-12.7)	(10.8- 12.3)	(10.5-13)	
							0.3†

Table 3 continued. Haemostatic variables, median (range), after infusion of each of the 3 treatments. Treatment A = HES (Voluven®) bolus of 10 ml/kg. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg.

Variable	Treatment	Baseline	1 hr	6 hr	12 hr	24 hr	*P* value *
aPTT (s)	A	43.50 (36.4-48.8)	37.55* (33.8-43)	44.10 (37.1- 46.6)	43.80 (36.6- 54.4)	41.20 (36.7- 42.3)	0.0002
	B	43.40 (37.4-45.3)	35.25* (33.6-40.1)	41.80 (37.7- 45.4)	41.45 (34.9- 54.6)	40.30 (36.8- 47.6)	0.03
	C	43.30 (38.4-47.6)	39.00 (34.5-46.8)	43.65 (36.7- 48.5)	43.25 (39.2- 47.7)	42.70 (36.6- 49.9)	0.78
							0.54†
Fibrinogen (g/L)	A	1.90 (1.58-2.59)	1.60* (1.38-1.99)	1.63* (1.61-1.84)	1.80 (1.53-2.07)	1.96 (1.67-2.37)	0.0033
	B	1.88 (1.38-2.3)	1.70 (1.49-2.06)	1.84 (1.61-2.25)	1.92 (1.68-2.14)	1.99 (1.91-2.47)	0.09
	C	1.84 (1.62-2.08)	1.48* (1.36-1.61)	1.69* (1.47-1.91)	1.79 (1.61-2.1)	2.09* (1.95-2.57)	<0.0001
							0.1†

***Based on mixed effects linear model with comparisons over time within each treatment group. Medians with asterisks are significantly different than baseline after Bonferroni correction of *P* values for multiple *post- hoc* comparisons. †Effect of treatment over all time points. Treatments without superscripts in common are significantly different after Bonferroni correction of *P* values for multiple *post- hoc* comparisons.**

Renal indices

No abnormalities were detected on microscopic USE at any time point for any horse. USG values remained consistent with normal renal function for each horse throughout the study.

Creatinine concentration remained within normal range at all time points throughout the study (Fig 10; Table 4). No significant difference was observed between pre- and post-study values (median pre-study: 138.0 µmol/l [range 117-150 µmol/l]; median post-study 134.5 µmol/l [range 120-147 µmol/l] $P = 0.5$). For all renal variables post study values were defined as those obtained 7 days after administration of the final treatment.

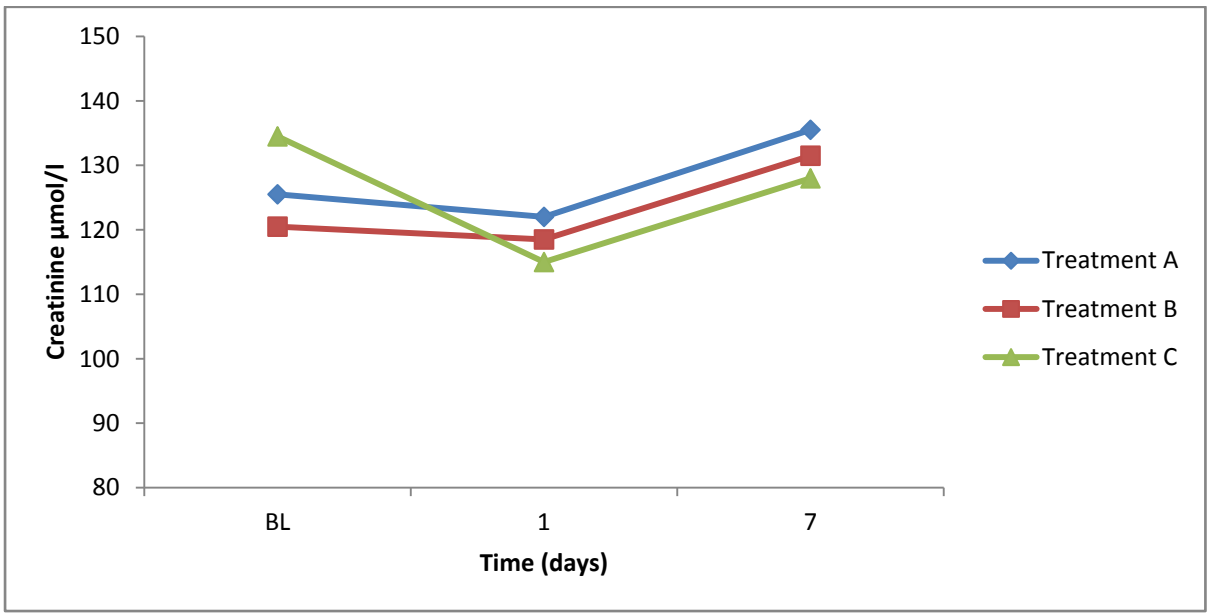

Figure 10. Effect, median of each of the 3 different treatments on creatinine over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt.

Table 4. Descriptive creatinine results, median (range), after infusion of each of the 3 treatments. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt.

Variable	Treatment	Baseline	1 day	7 days
Creatinine (µmol/L)	**A**	125.5 (113-136)	122.0 (116-129)	135.5 (114-147)
	B	120.5 (109-130)	118.5 (101-136)	131.5 (121-142)
	C	134.5 (103-149)	115.0 (92-129)	128.0 (120-145)

There was no significant difference between pre- and post-study UPC values (Fig 11; Table 5; median pre-study: 0.11 [range 0.08-0.13]; median post study: 0.13 [range 0.08-0.25] $P = 0.3$). UPC values were higher at 24 h relative to the baseline values after administration of treatment B and C ($P = 0.05$ and $P = 0.03$ respectively).

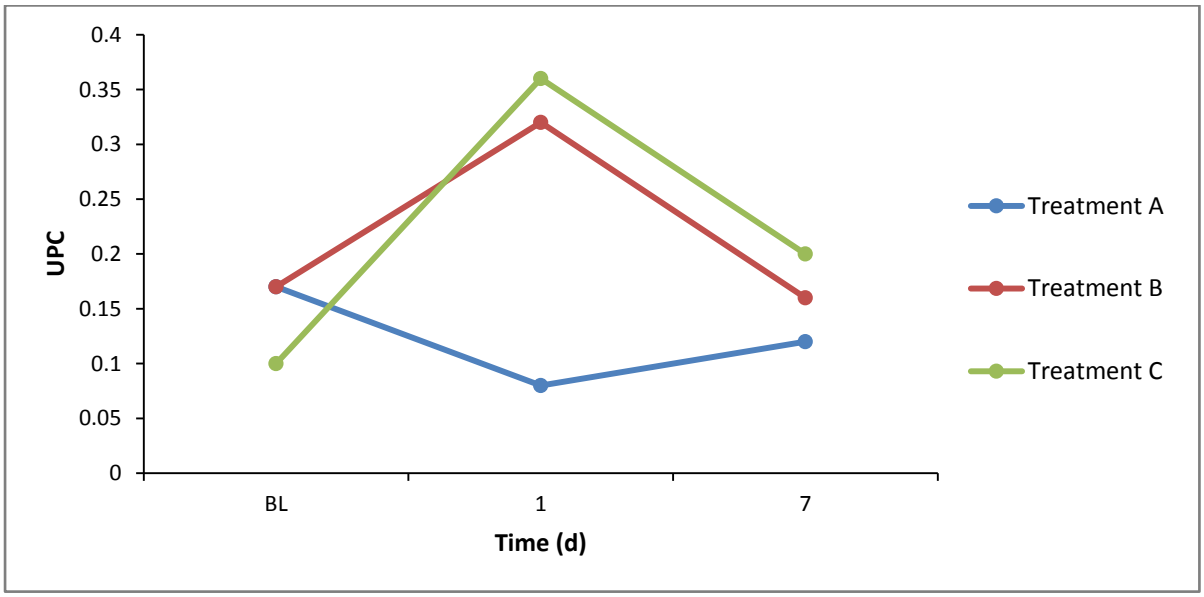

Figure 11. Effect, median, of each of the 3 treatments on UPC over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt. BL= baseline.

There was no significant difference between pre- and post- study UGC values (Fig 12; Table 5; median pre-study: 12 [range 6-22]; median post study: 10 [range 6-14] *P* = 0.6).

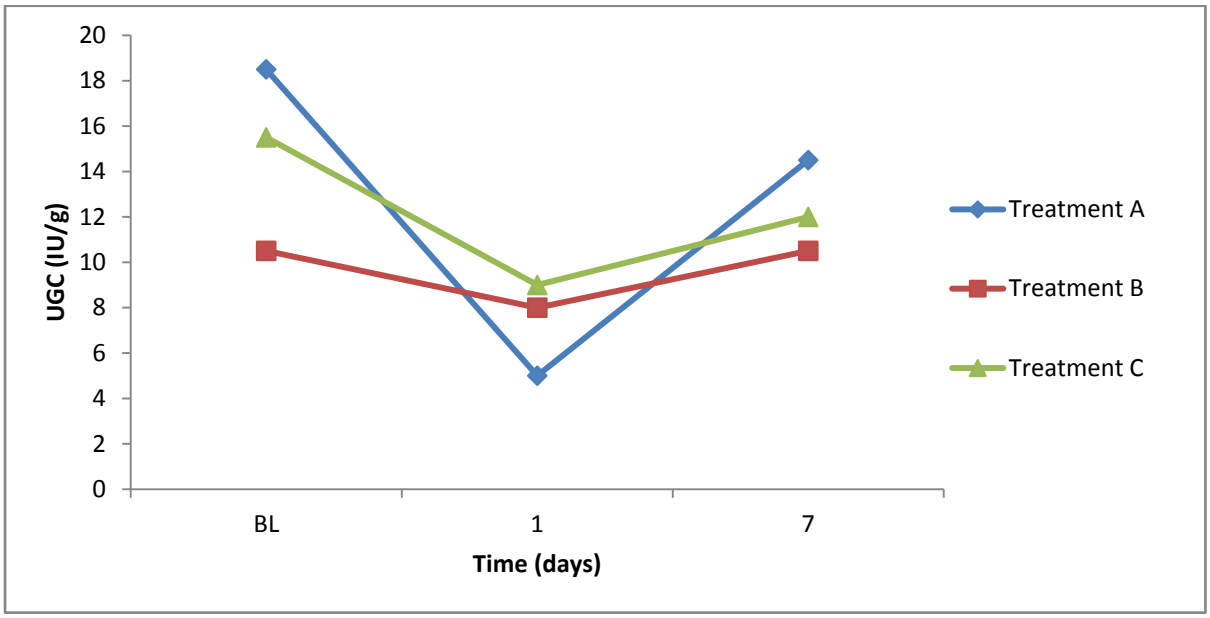

Figure 12. Effect, median, of each of the 3 treatments on UGC over time. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt. BL= baseline.

Table 5. Descriptive UPC and UGC results, median (range), after infusion of each of the 3 treatments. Treatment A = HES (Voluven®) bolus of 10 ml/kg bwt. Treatment B = MFG (Gelofusine®) bolus of 10 ml/kg bwt. Treatment C = MFG (Gelofusine®) bolus of 20 ml/kg bwt.)

Variable	Treatment	Baseline	24 h	1 week
UPC	**A**	0.2 (0.1-0.3)	0.1 (0.1-0.2)	0.1 (0.1-0.2)
	B	0.2 (0.1-0.5)	0.3 (0.2-0.4)	0.2 (0.1-0.3)
	C	0.1 (0.1-0.1)	0.4 (0.2-0.9)	0.2 (0.1-0.5)
UGC (IU/g)	**A**	18.5 (8-53)	5.0 (2-17)	14.5 (6-34)
	B	10.5 (6-29)	8.0 (6-23)	10.5 (8-53)
	C	15.5 (8-34)	9.0 (5-42)	12.0 (7-29)

DISCUSSION

This study identified MFG at dosages of 10 and 20 ml/kg bwt i.v. as a potential alternative to HES for volume expansion and augmentation of COP in equine patients. Neither product induced detectable renal injury or a deficiency in haemostasis. No clinical signs of anaphylaxis were observed during the study.

In the present study administration of HES resulted in a significant decrease in haematocrit until 6 h post infusion and a comparable effect was observed with the same dose of MFG. While direct comparisons with other studies cannot be made due to different sampling times, similar effects of HES on haematocrit in horses have been previously described [6, 28]. Similar results were reported for healthy humans, where the effects on volume expansion were comparable after administration of equal volumes of 6% HES or 4% MFG over a 6 h period post infusion [3]. Additionally, in the present study the duration of effect of MFG on haematocrit following a 20 ml/kg bwt dose was longer than that following a 10 ml/kg bwt dose, suggesting a dose-dependent effect of MFG on haemodilution.

Platelet count was only significantly decreased from baseline following the administration of treatment C, consistent with the greater effects on haemodilution observed. Nonetheless, platelet count remained within laboratory reference range (>100 x 10^9/L) throughout the study. In the present study TSP decreased following a 10 ml/kg bwt dose of HES until 12 h post infusion, longer than the 6 h reported previously [28]. The reason for this difference is unclear, but may be due to the use of different techniques for measuring total solids. In the present study, administration of MFG at 10 ml/kg bwt had less effect on TSP than an equivalent dose of HES. A possible explanation for this could be that the proteins contained in the MFG may have been detected by the serum protein assay. The biuret method of protein quantification (used in this study) has been demonstrated to detect MFG in urine [61], although interference with serum protein measurement has not previously been described. The 4% MFG administered in the present study was subsequently analysed using the biuret method, producing a protein measurement of 31 g/L when undiluted. Without consideration of other physiological factors, theoretically in a normovolaemic animal receiving a 10 ml/kg bwt dose, this equates to an approximate increase of 5 g/L

in total serum protein. The dose of 10 ml/kg bwt MFG had similar effects on TSP to the 20 ml/kg bwt dose. This could be explained by a greater contribution to TSP by the greater dose of gelatin given, with a concurrent greater dilutional affect due to the greater dose of gelatin being given.

Following HES administration COP was significantly increased from baseline for 24 h, consistent with the findings of previous studies [6, 28]. MFG also significantly increased COP for 24 h at both doses, with no significant differences observed between the treatment groups. Data comparing the duration of effect between these two colloids on COP in animal species have not previously been published. Limited clinical data are available comparing the use of these two colloids for the augmentation of COP under specific circumstances in human ICU patients [12, 13], where colloid doses were variable and concurrent intravenous crystalloids were administered. In these human studies COP was observed to decrease comparably during abdominal aortic aneurysm surgery in patients receiving either HES or MFG [12], but a greater decrease in COP was observed in patients receiving MFG compared with HES during cardiopulmonary bypass [13]. In contrast to the postulated dose-dependent effect on haemodilution, a dose-dependent effect on COP was not observed following MFG administration in the present study. The number of molecules per unit of space determines the osmotic pressure of a colloidal solution [1]. It would therefore be anticipated that an increase in colloid dose would lead to an increased colloid osmotic pressure. This effect has been observed clinically following the administration of different doses of hydroxyethyl starch to healthy ponies [8]. In a more recent study with healthy ponies [5], a dose-dependent effect of HES on COP was reported for doses of 10 ml/kg bwt and 40 ml/kg bwt. A dose-dependent increase in COP was also observed at 4 and 8 h post-treatment when 2 different concentrations of a MFG was administered to pigs with septic shock [22]. While the data from the present study represent the direct effects of these two colloids on COP in ponies under controlled circumstances, further investigation is required to evaluate the effects in sick equine patients and in particular, those receiving concurrent i.v. crystalloid fluids.

TEG is a viscoelastic, whole blood-based assay that analyses both the cellular and soluble components of the coagulation process. Compared with other methods of assessing coagulation it provides a more global assessment of the haemostatic system and detects changes in the viscoelastic

properties of a blood clot, from its formation through to fibrinolysis. TEG has previously been used to assess coagulability in horses with a range of clinical conditions [53-55]. No significant differences from baseline were observed for R, K, α-angle, MA, G, LY30 or LY60 in any of the treatment groups and the findings for HES were consistent with those previously reported in equids [28, 62].

PT and aPTT values are considered clinically significant if their levels change by 20% or greater [63], although there is little evidence to support this cut off value. A statistically significant increase in median PT from baseline was recorded after treatment A and after treatment C, although these values were < 20%. Median aPTT was transiently decreased from baseline after treatments A and B, but no significant difference in median aPTT was seen after treatment C. A decrease in aPTT of ≥ 20% was detected in 1 pony after treatment C and 3 ponies after treatment B. A previous study reported a decrease in both PT and aPTT after administration of hetastarch [64]. In contrast, a later study identified an increase in PT and aPTT after administration of either hetastarch or tetrastarch [6]. The finding that all TEG parameters remained within normal limits in the present study suggests that the changes in aPTT and PT had no overall effect on whole blood coagulation. While the use of other tests of coagulation alongside TEG allows for effects on individual components of coagulation to be detected, in the presence of normal TEG results, they are considered unlikely to be of clinical significance.

Fibrinogen concentration remained within the laboratory reference range for all treatments and time points, therefore the significant differences detected from baseline are not considered to be clinically significant.

A high frequency of mild phlebitis (39%) was observed during this study, possibly related to the large gauge of catheter used. No association between treatment type or study week was observed. There is no reported association between either HES or MFG and phlebitis. Theoretically it is possible that the presence of phlebitis with inherent endothelial activation may have increased the coagulability of the affected ponies' blood. Furthermore, small amounts of tissue factor are inadvertently infused into the first sample during blood collection via venepuncture. In order to pre-empt this as a potential confounding factor, serum samples were collected prior to the citrate tube for coagulation analyses. As no association between treatment type and phlebitis was identified and no significant effects on

haemostasis were observed, these episodes are not considered to have biased the results of the study.

Limited data are available regarding the normal range for UPC in the horse. A range of 0.03 to 0.93 (median 0.11) has previously been described for 17 horses [65] and in the present study UPC values remained within this range. Consistent with a previous report [65] which described variability in UPC ratios throughout the day, UPC values were variable throughout the present study for all horses. Significant differences in UPC compared with baseline were observed during the study, but there were no long-term effects. Four out of the 6 horses had their highest recorded UPC 24 h after the administration of treatment C. Modified fluid gelatins have been shown to reduce proximal tubular reabsorption of low-molecular weight proteins such as globulins [37, 38], which can lead to increased levels of these proteins in the urine. This has been demonstrated in the absence of N-acetyl-beta-D-glucoaminidase, a proximal tubular lysosomal enzyme used to screen for proximal tubular cell damage in human patients. These findings were consistent with transiently altered proximal tubular function in the absence of tubular damage, as has been described in humans [37]. A marked pseudoproteinuria has also been described following modified fluid gelatin administration and is a direct result of cross-reactivity of the gelatins with urine proteins in certain assays used for protein measurement [39]. The benzethonium chloride tubidimetry assay, used in the current study is not significantly affected by the presence of modified fluid gelatins [66], so it is unlikely that this contributed to the increased UPCs observed. Current assays validated for the assessment of equine proteinuria cannot differentiate between albumin, low molecular weight plasma proteins and mucoproteins. It was therefore not possible to determine the origin of the urinary protein in this study.

Although there are limited publications describing UGC values in equids, less than 25 IU/g is considered to be normal [67, 68]. Values greater than 100 IU/g have been reported after prolonged gentamicin treatment and are considered likely to be associated with tubular damage [58], while values between 25 and 100 IU/g cannot be definitively interpreted. UGC values remained less than 100 IU/g throughout the present study, but a number were between 25 and 100 IU/g. The present study was performed using normal ponies, the data cannot therefore be directly extrapolated to

equids with increased vascular permeability. Further investigation is required to determine the relative size and duration of any clinical effects in compromised equids. Another limitation of the study was the small number of animals used. This could have affected the power of the study.

CONCLUSIONS

In conclusion, MFG can be considered as an alternative to HES for volume expansion and oncotic support in equids. No significant differences in haematocrit or COP were observed between equal doses of MFG and HES. Haemodilution following MFG was dose-dependent, with both a larger and longer duration of effect being observed following a 20 ml/kg bwt versus a 10 ml/kg bwt dose. No dose-dependent changes were observed for COP. Neither MFG nor HES were associated with clinically significant adverse effects on haemostasis or renal parameters at the described doses.

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