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# An in vitro study of canine cryopoor plasma to correct vitamin K–dependent coagulopathy in dogs

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## Abstract

**Objective:** To compare the efficacy of fresh frozen plasma (FFP) with cryopoor plasma (CPP) to treat vitamin K–dependent factor deficiency in a canine in vitro setting.

**Design:** In vitro laboratory study.

**Setting:** University veterinary medical teaching hospital.

**Animals:** Seven units of FFP and 6 units of CPP from unique canine donors from the university veterinary blood bank.

**Interventions:** Canine FFP was adsorbed by oral barium sulfate suspension to mimic vitamin K–dependent coagulopathy. A sequential mixing study was completed by adding FPP or CPP to the adsorbed plasma. Measurements of prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and factor activities of factors II, VII, and IX (FII, FVII, and FIX) were compared between the 2 treatment groups.

**Measurements and main results:** When comparing the sequential addition of CPP or FPP to adsorbed plasma, the following had no statistical significance: PT ($P = 0.94$), aPTT ($P = 0.66$), FII ($P = 0.05$), and FIX ($P = 0.90$). There was a dose-dependent decrease with PT and aPTT and a dose-dependent increase with FII and FIX. In contrast, after the addition of either CPP or FFP, there was a significant difference between the treatment groups for the concentration of fibrinogen ($P = 0.005$) and activity of FVII ($P = 0.044$), with FFP resulting in a greater concentration of fibrinogen and CPP resulting in a greater concentration of FVII. Measurements of factor X (FX) were initially included in the study but were later excluded because FX appeared to be continually adsorbed even after the addition of CPP or FFP.

**Conclusions:** CPP partially corrected the coagulation times and concentration of vitamin K–dependent coagulation factors to the same degree as FFP. CPP, generally less expensive than FFP, may provide an alternative treatment option for vitamin K–dependent coagulopathies, although in vivo testing is needed.

KEYWORDS
canine, coagulation, fresh frozen plasma, rodenticide

**Abbreviations:** aPTT, activated partial thromboplastin time; ARI, anticoagulant rodenticide intoxication; CPP, cryopoor plasma; FFP, fresh frozen plasma; FII, factor II; FIX, factor IX; FV, factor V; FVII, factor VII; FVIII, factor VIII; FWB, fresh whole blood; FX, factor X; PT, prothrombin time

## 1 | INTRODUCTION

In veterinary emergency clinics, various coagulopathies are one of the most commonly seen disorders. Although there may be many causes for these coagulopathies, one of the concerning coagulopathies includes vitamin K–dependent factor deficiencies. In severe cases, this can lead to life-threatening hemorrhages, such as hemothorax or hemoabdomen, resulting in death.[1,2] Anticoagulant rodenticides are a particular concern because they are one of the most commonly ingested toxins in veterinary medicine.[3,4]

Anticoagulant rodenticide ingestion (ARI) prevents the recycling of vitamin K, which leads to decreased concentrations of coagulation factors II, VII, IX, and X (FII, FVII, FIX, and FX).[1,2,5] Traditional treatment for vitamin K–dependent factor deficiency includes fresh whole blood (FWB) and fresh frozen plasma (FFP) because they contain all coagulation factors, including those affected by the rodenticide's vitamin K antagonist mechanism.[2,5] Cryopoor plasma (CPP) is the supernatant remaining from FFP processing to produce cryoprecipitate.[6] In a recent study, CPP contained similar amounts of albumin, FII, FVII, FIX, and FX compared to FFP.[7] CPP can be much less expensive than FFP and thus may offer a more cost-effective treatment option for veterinarians and clients, in addition to improving utilization of scarce blood resources through targeted blood product use. For example, at a university canine blood bank, 1 unit of CPP currently costs 38% less than a unit of FFP. It is currently unknown whether CPP can be used instead of FFP to normalize coagulation times in ARI dogs.

Our study aims to determine whether CPP may be considered as a potential treatment option for ARI by comparing its ability with that of FFP to shorten coagulation times and replace activities of coagulation factors in canine plasma depleted ex vivo of vitamin K–dependent coagulation factors. We hypothesized that CPP would correct abnormal coagulation times and factor activities at doses similar to FFP in an in vitro model of anticoagulant rodenticide intoxication.

## 2 | MATERIALS AND METHODS

### 2.1 | Plasma

FFP and CPP were produced by the UC Davis Veterinary Medical Teaching Hospital Blood Bank. This blood was collected from healthy, screened dogs in a community blood donor program. Donors were between 1 and 8 years old and weighed greater or equal to 25 kg with a normal physical examination and complete bloodwork at entry into the program (CBC, biochemistry panel, von Willebrand's factor, blood typing, and antigens for *Dirofilaria immitis*, *Brucella canis*, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Bartonella* spp., *Ehrlichia canis*, *Rickettsia* spp., *Mycoplasma haemofelis/haemocanis*, *Leishmania*, *Babesia* spp., *Mycoplasma haematoparvum*, and *Babesia conrada*).[8] In this study, plasma was collected from non-Greyhound mixed breed dogs that fit the previously mentioned criteria. Although there may be some variability between donors with the concentration of coagulation factors or fibrinogen, coagulation values for each sample have been measured prior to experimentation and are included in the results. For a typical donation, blood was collected from the jugular vein in sitting dogs using a 16-Ga needle. Approximately 450 mL of blood was collected at a time using a vacuum system.[7]

For processing of FFP, a minimum volume of 425 mL FWB was collected in order to ensure proper citrate* to blood ratios at 1.4:10. Using a centrifuge,† the FWB was spun at 5,000 x *g* at 4°C for a total run time of 8 minutes. After centrifugation, the centrifuged FWB was placed in a plasma extractor, separating the plasma into the attached bag with no contents (an additional bag will have an additive for the pRBCs). Typically, 225 to 250 mL of plasma is yielded from 1 FWB donation. The bag with the plasma was then sealed off using a tube sealer and added to an additional bag using a tube welder for further processing. Plasma was stored in the freezer at -18°C or lower until the FFP was completely frozen.

For processing of CPP, the FFP is partially thawed at 4°C to a "slushy" consistency.[6] The FFP is then centrifuged at 5,000 x *g* for 17 minutes. The cryoprecipitate will precipitate and adhere to the sides of the bag. Using a plasma press, the next step is to express the supernatant into the attached bag. The plasma press works by releasing a hinged plate and applies pressure to the bag with the centrifuged whole blood. Ninety percent of the supernatant is expressed into the attached satellite bag and becomes the CPP, and the 10% remaining in the original bag is the cryoprecipitate.[9] Both FFP and CPP were frozen until the first phase of the study.

### 2.2 | Thawing

FFP and CPP were thawed to 37° by dry tempering.‡ An infrared sensor detected the temperature of the plasma during the process until the goal temperature was met. Plasma was gently agitated on a rocking plate to ensure homogeneity of thawing. Up to 3 units of plasma were thawed at a time.

### 2.3 | Adsorption of plasma

A barium protocol§ was used to deplete plasma of vitamin K–dependent coagulation factors. To adsorb the plasma of coagulation factors II, VII, IX, and X, oral barium sulfate** (200 mg $BaSO_4$ per mL of FFP) was mixed into 1 unit of FFP. The solution was mixed for 30 minutes in a 37°C water bath and inverted 10 times every 5 minutes. Then the barium was precipitated out by centrifugation at 1,500 rpm for 15 minutes. The supernatant was carefully removed from the tube and was frozen for at least 24 hours to ensure full precipitation of the barium sulfate. The plasma was slowly thawed in a 37°C water bath and centrifuged again at the same rate. The supernatant was removed and served as the base of all adsorbed plasma for this investigation. To confirm factor depletion, coagulation tests†† (prothrombin time [PT], activated partial thromboplastin time [aPTT], and fibrinogen) were

completed in the clinical laboratory at the UC Davis Veterinary Medical Teaching Hospital within a few hours of adsorption, while the rest of the samples were shipped overnight on dry ice to the Comparative Coagulation Lab at the Cornell University Animal Health Diagnostic Center to measure the activities of factors II, VII, IX, and X as described below.

## 2.4 | Mixing study

Calculations were conducted to approximate clinical doses of plasma transfused to ARI dogs. The plasma per body weight in dogs is estimated at 51.5 mL/kg.[10] To treat severe cases of ARI, the recommended starting dose for FFP transfusions is 9 mL/kg of FFP,[5] although clinically, doses often range between 10 and 20 mL/kg of FFP depending on the individual patient. This is a 5:1 to 5:2 ratio of plasma volume in the patient to amount of plasma transfused for treatment. Therefore, for a 2-mL barium adsorbed plasma sample, 0.4 to 0.8 mL of FFP equates to the standard clinical doses used to normalize the PT. An escalating dosage study was created to assess for optimized proportional volume. For each set of trials, 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of either CPP or FFP were added to a 2-mL aliquot of adsorbed plasma using a pipette. One milliliter of this mixture was collected for immediate measurement of PT, aPTT, and fibrinogen.[††] The remaining plasma was frozen at −80C° and then shipped overnight on dry ice for batch analysis of coagulation factor activities. Six different units of FFP and 6 different units of CPP were used for each set of trials.

## 2.5 | Factor analyses

Coagulant activities of factors II, VII, and X (FII:C, FVII:C, FX:C) were measured using a 1-stage PT technique, a rabbit brain thromboplastin reagent,[‡‡] and an automated[††] or semi-automated[§§] mechanical endpoint clot detection instrument. The assays were configured with either human substrate deficient plasma[***] or an adsorbed, artificially depleted bovine plasma for determination of FX:C. Factor IX activity was measured in a 1-stage activated PTT assay, using a semi-automated clot detection instrument,[***] a kaolin and ellagic acid–activating reagent,[†††] and human factor IX–deficient plasma.[‡‡‡]

## 2.6 | Data analysis

Descriptive statistics were completed to look for differences in coagulation analyses and factor activities between the CPP and FFP groups at the different dose levels using multilevel mixed-effect linear regression. The individual coagulation or factor data were set as the dependent variable. The categorical variable grouping an item into either the CPP or FFP group and the ordinal variable of dose level were set as the independent variables in the model. The variable that categorized the unit of CPP or FFP used was added to the model as a random effect parameter. The overall model was checked for significance, and the model was also checked to see if including the random effect parameter improved the model over a simple linear regression. Statistical significance was set at $P < 0.05$. Statistics were performed using a commercially available software program.[§§§]

**TABLE 1** Reference and baseline pre-mixing values for coagulation tests and factors measured in healthy canine CPP (n = 6)

| Parameter | Reference Value | Mean | SD | Range | Culler et al[4] Mean |
|---|---|---|---|---|---|
| PT (s) | 11-15.5 | 9.8 | ±0.78 | 9-11.1 | N/A |
| aPTT (s) | 8.5-15.5 | 15.6 | ±1.02 | 82-130 | N/A |
| Fibrinogen (g/L) | 1.50-4.90 | 1.04 | ±0.21 | 0.82-1.3 | 0.53 |
| FII (%) | 50-150 | 109.8 | ±21.67 | 87-139 | 123.22 |
| FVII (%) | 50-150 | 100.7 | ±34.89 | 54-159 | 101.75 |
| FIX (%) | 50-150 | 73.8 | ±16.40 | 60-105 | 67.9 |
| FX (%) | 80-175 | 103.5 | ±49.49 | 58-177 | 121.8 |

PT, prothrombin time; aPTT, activated partial thromboplastin time; FII, factor II; FVII, factor VII; FIX, factor IX; FX, factor X; N/A, not applicable.
For the ease of comparison of similar measurements from a previous investigation (Culler et al[4]), data are provided in the shaded column (n = 10).

**TABLE 2** Reference and baseline pre-mixing values for coagulation tests and factors measured in healthy canine FFP (n = 6)

| Parameter | Reference Value | Mean | SD | Range | Culler et al[4] Mean |
|---|---|---|---|---|---|
| PT (s) | 11-15.5 | 8.6 | ±0.74 | 7.4-9.5 | N/A |
| aPTT (s) | 8.5-15.5 | 12.2 | ±1.12 | 10.6-13.8 | N/A |
| Fibrinogen (g/L) | 1.50-4.90 | 1.48 | ±0.35 | 1.09-1.98 | 1.19 |
| FII (%) | 50-150 | 100.5 | ±5.47 | 96-108 | 120.33 |
| FVII (%) | 50-150 | 109.2 | ±30.37 | 76-156 | 118.8 |
| FIX (%) | 50-150 | 109.7 | ±30.45 | 76-161 | 84.7 |
| FX (%) | 80-175 | 92.7 | ±28.63 | 62-134 | 145 |

PT, prothrombin time; aPTT, activated partial thromboplastin time; FII, factor II; FVII, factor VII; FIX, factor IX; FX, factor X; N/A, not applicable.
For the ease of comparison of similar measurements from a previous investigation (Culler et al[4]), data are provided in the shaded column (n = 10).

# 3 | RESULTS

## 3.1 | Baseline values

PT, aPTT, fibrinogen, and activities of FII, FVII, FIX, and FX were measured for both CPP and FFP (n = 6). For CPP, the mean PT = 9.82 seconds (±0.78), aPTT = 15.57 seconds (±1.02), fibrinogen = 1.04 m/L (±0.21), FII = 109.83% (±21.67), FVII = 100.67% (±34.89), FIX = 73.83% (±16.40), and FX = 103.50% (±34.89) (Table 1).

For FFP, the mean PT = 8.6 seconds (±0.74), aPTT = 12.17 seconds (±1.12), fibrinogen = 1.48 g/L (±0.35), FII = 100.5% (±5.47), FVII = 109.17% (±30.37), FIX = 109.67% (±30.45), and FX = 92.67% (±28.63) (Table 2).

## 3.2 | Adsorbed plasma mixing results

### 3.2.1 | Prothrombin time

There was no significant difference in PT between CPP and FFP groups with the addition of 0 to 1 mL of either CPP or FFP to 2-mL aliquots of adsorbed plasma ($P = 0.94$). However, there was a significant dose-dependent difference ($P < 0.0001$) that was negatively correlated with the PT (coefficient -8.75; 95% CI, -10.04 to -7.47), meaning that an increase in dose of either CPP or FFP resulted in a shortening of the PT. Sequentially, from lowest to highest volume of CPP mixed into adsorbed plasma, the median PT was 18.9 seconds (16.4-21.4), 14.4 seconds (13.1-15.9), 13.2 seconds (11.2-14.9), 12.1 seconds (11.1-13.2), and 11.9 seconds (10.7-12.7). For FFP, the median PT was 19.7 seconds (17.1-22.1), 14.7 seconds (12.6-15.5), 12.9 seconds (11.4-13.5), 12.1 seconds (10.8-12.6), and 11.4 seconds (10.5-12.2) (Figure 1).

### 3.2.2 | Activated partial thromboplastin time

Similarly, there were no significant differences in aPTT between the 2 groups with the addition of 0 to 1 mL of either CPP or FFP to 2-mL aliquots of adsorbed plasma ($P = 0.66$). However, there was a significant dose-dependent difference ($P < 0.0001$) with a negative coefficient with aPTT (coefficient -90.9; 95% CI, -97.97 to -83.83). Sequentially, the median aPTT for CPP = 140.0 seconds (139.2-140), 127.4 seconds (95.4-131.3), 100.1 seconds (75.8-111.5), 83.5 seconds (58.9-88.6), and 73.6 seconds (54.2-77.1). The median aPTT for FFP was 140.0 seconds (133.5-140), 114.0 seconds (87.2-137.8), 87.9 seconds (69.4-113.6),74.7 seconds (57.3-95.4), and 63.8 seconds (51-84.3) (Figure 1).

## 3.3 | Fibrinogen

After sequential addition of 0 to 1 mL of either CPP or FFP to 2-mL aliquots adsorbed plasma, there was a significant difference in the concentration of fibrinogen between CPP and FFP ($P = 0.005$), as well as a significant dose-dependent difference ($P = 0.004$) in the concentration of fibrinogen for both plasma products. The coefficient was positive (coefficient 5.92; 95% CI, 1.93 to 9.90), with fibrinogen increased in response to an increased dose. From lowest to highest amount of added CPP, the median fibrinogen concentrations were 1.055 g/L (1.04-1.09), 1.075 (1.01-1.13), 1.055 (1.00-1.12), 1.075 (0.97-1.13), and 1.035 (0.96-1.14). Meanwhile, sequentially, the addition of FFP resulted in the median fibrinogen concentrations of 1.105 g/L (1.06-1.13), 1.12 (1.07-1.22), 1.15 (1.08-1.27), 1.13.5 (1.07-1.33), and 1.205 (1.07-1.39) (Figure 1).

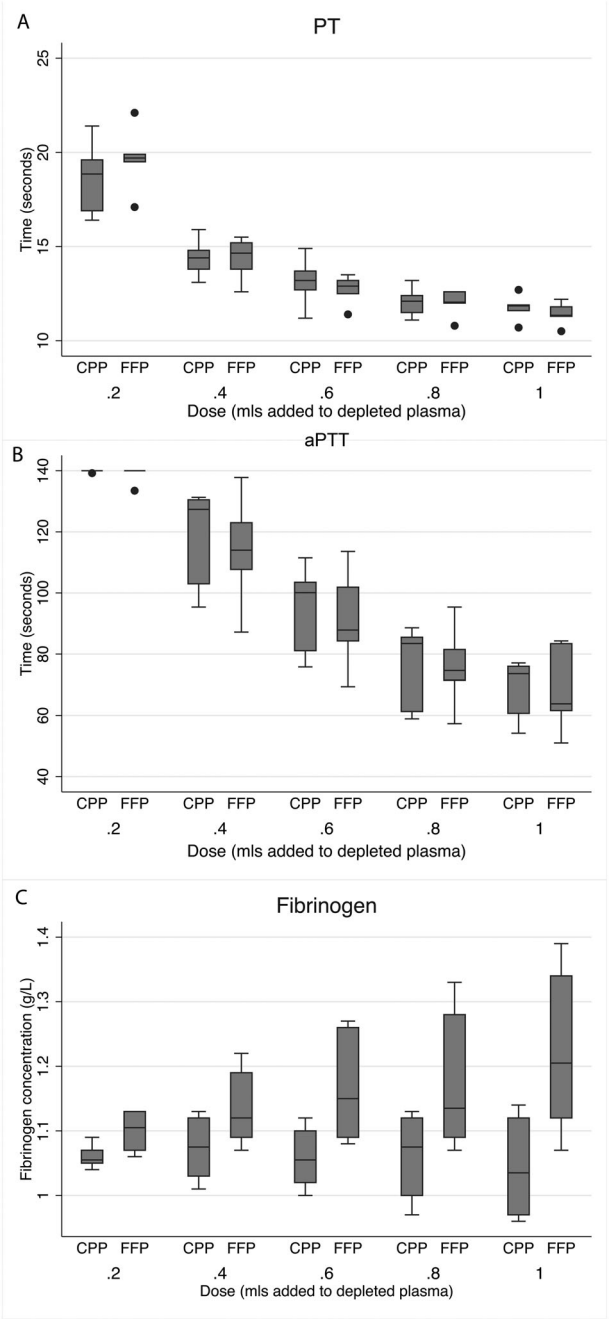


**FIGURE 1** The effects on PT (A), aPTT (B), and fibrinogen (C) with increasing concentrations of CPP and FFP in barium-adsorbed plasma. Time (in s) is shown on the *y*-axis, and dose of added blood product (in mL) is indicated on the *x*-axis. The box indicates interquartile range (75% upper and 25% lower limits), the bar indicates the median value, the whiskers represent the minimum and maximum value up to 1.5 times the upper and lower quartile, and the dots indicate outlying values. PT, prothrombin time, aPTT, activated partial thromboplastin time; CPP, cryopoor plasma; FFP, fresh frozen plasma

### 3.4 | Factor II

There was no significant difference in FII between the 2 dosing groups with the increasing addition of 0 to 1 mL of CPP or FFP to 2-mL aliquots of adsorbed plasma ($P = 0.05$). There was a significant dose-dependent difference ($P < 0.0001$) with a positive coefficient (coefficient 31.04, 95% CI, 28.53 to 33.55) between factor activity and the dose of CPP or FFP. Sequentially, from lowest to highest concentration of CPP mixed into adsorbed plasma, the median concentration of FII was 10% (2-13), 17% (12-20), 22.5% (18-30), 29.5% (24-40), and 37.5% (25-40). With the addition of FFP, the median concentrations of FII were 8% (7-9), 15% (11-17), 19% (16-24), 24.5% (20-26), and 33.5% (26-39) (Figure 2).

### 3.5 | Factor VII

There was a significant difference between the addition of 0 to 1 mL of CPP and FFP to 2-mL aliquots of adsorbed plasma with FVII activity ($P = 0.044$). For both, there was a difference based on increasing dose ($P < 0.0001$). The coefficient is positive (coefficient 35.25, 95% CI, 31.20 to 39.30), where increases in the dose also resulted in increases in FVII activity. The addition of CPP sequentially resulted in the median concentrations of FVII of 9% (6-14), 21.5% (12-28), 26% (12-38), 35.5% (26-39), and 43.5% (31-55), while the addition of FFP resulted in the median concentrations of 7% (3-12), 13% (11-21), 20.5% (13-21), 27.5% (18-40), and 25.5% (23-52) (Figure 2).

### 3.6 | Factor IX

For FIX, there was no significant difference between the addition of 0 to 1 mL of CPP and FFP to 2-mL aliquots of adsorbed plasma ($P = 0.90$). There was a significant dose-dependent difference ($P < 0.0001$, coefficient 18.83, 95% CI, 17.80 to 19.86). The addition of CPP sequentially resulted in the median concentrations of FVII of 11% (10-13), 14.5% (14-19), 19.5% (18-24), 22.5% (20-28), and 25.5% (23-30), while the addition of FFP resulted in the median concentrations of 11% (9-12), 16% (13-18), 19.5% (17-23), 23% (20-27), and 24% (24-31) (Figure 2).

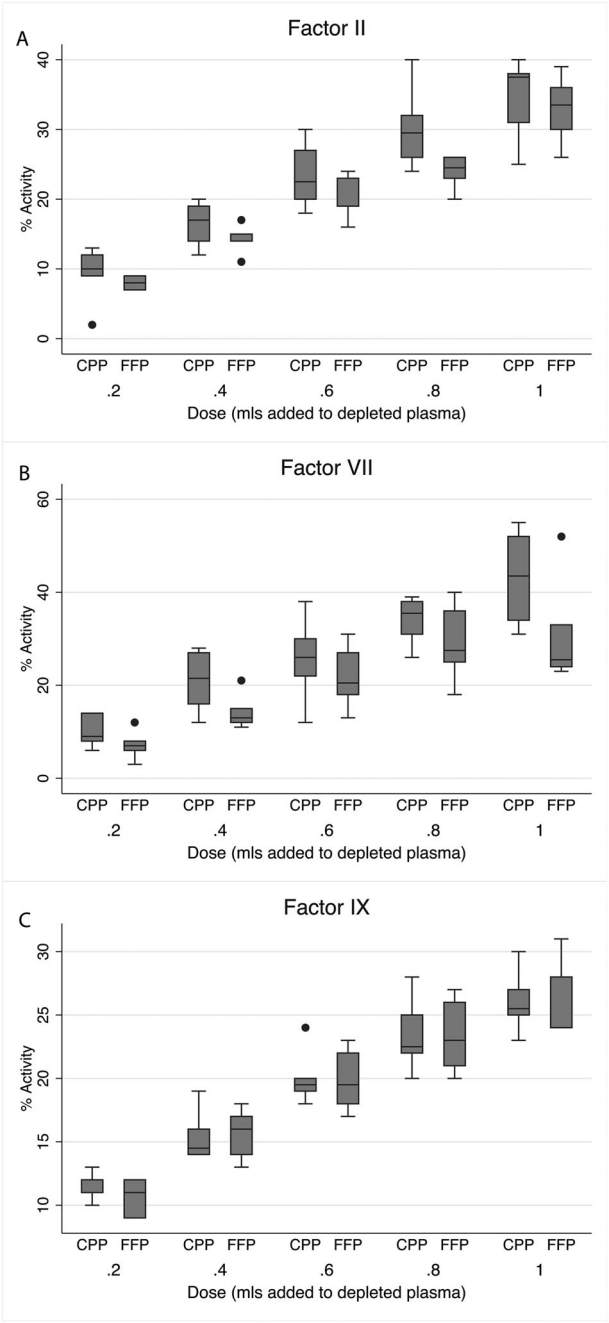


**FIGURE 2** The effects on the activities of factor II (A), factor VII (B), and factor IX (C) with increasing concentrations of CPP or FFP in barium-adsorbed plasma. The box indicates interquartile range (75% upper and 25% lower limits), the bar indicates the mean value, the whiskers represent the range, and the dots indicate outlying values. CPP, cryopoor plasma; FFP, fresh frozen plasma

## 4 | DISCUSSION

Anticoagulant rodenticides are a common class of pesticides used to control unwanted rodent populations. ARI is one of the most common intoxications seen in emergency veterinary clinics.[3,4] According to ASPCA Animal Poison Control Center, anticoagulant rodenticides were the eighth most ingested toxin in 2017[3] and seventh most ingested toxin in 2018.[4] The mechanism of action of ARI involves inhibition of vitamin K epoxide reductase and vitamin K reductase in the liver. These enzymes play an essential role in recycling vitamin $K_1$ (Figure 3), although there is an alternative pathway that can bypass the inhibition of vitamin K reductase.[2,5,11] In the liver, vitamin K epoxide reductase activates vitamin K epoxide into its active form, vitamin $K_1$ or vitamin K hydroquinone, which is further processed into the cofactor required for post-translational modification of FII, FVII, FIX, and FX. Collectively referred to as the vitamin K–dependent factors, FII, FVII, FIX, and FX depend on this vitamin $K_1$–mediated gamma

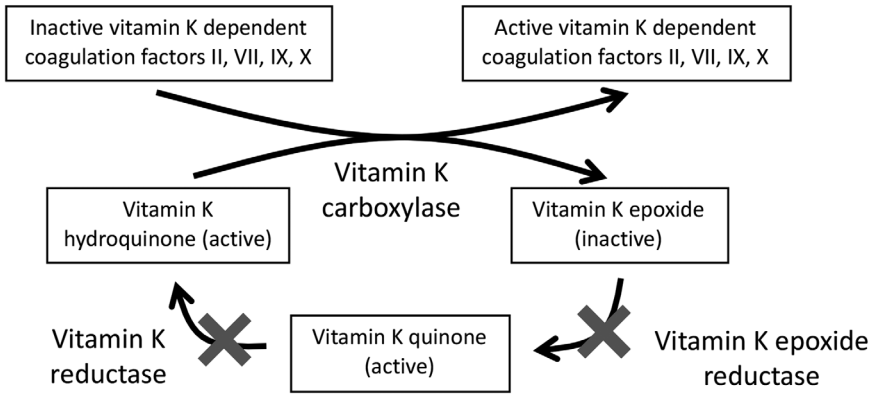


**FIGURE 3** The effects of ARI on the vitamin K–dependent coagulation factor activation system. In a normal patient, vitamin K epoxide is converted into vitamin K hydroquinone, or vitamin $K_1$, which is the active form that activates vitamin K–dependent factors FII, FVII, FIX, and FX using vitamin K carboxylase. In ARI, vitamin K epoxide reductase and vitamin K reductase are inhibited, preventing recycling and activation of vitamin K. The Xs indicate inhibition of that pathway by anticoagulant rodenticides. ARI, anticoagulant rodenticide intoxication; FII, factor II; FVII, factor VII; FIX, factor IX; FX, factor X

carboxylation step for their biological activity. In a rodenticide-intoxicated animal, there is a relative deficiency in active vitamin K, thus leading to a decrease in functional vitamin K–dependent coagulation factors that can lead to severe hemorrhage that is typically cavitary.[2,5,12] In severe cases, once bleeding has manifested (typically 3–5 days from ingestion), initial stabilization requires FFP or fresh whole blood (FWB) transfusions combined with hospitalized care and initiation of vitamin $K_1$.[5] Although FFP has been the gold standard treatment for severe ARI hemorrhaging, CPP has been occasionally used in clinics as well. In our study, for all coagulation tests and factors measured (PT, aPTT, FII, FIX), with the exception of fibrinogen and FVII activity, we found no significant difference between the sequential addition of CPP or FFP to factor-depleted canine plasma.

Due to the severity of hemorrhage, ARI in nontarget animals is a concern.[13] Accidental ingestion of rodenticide may occur due to direct ingestion of the substance, ingestion of feed contaminated by a rodenticide, or ingestion of poisoned rodents.[2] According to a study conducted in Europe, dogs are the most common companion animals seen for rodenticide toxicosis (20%), followed by cats (11%), and horses (1.1%).[14] Initial ingestion of anticoagulant rodenticide is followed first by a decrease of coagulation FVII, due to its shorter half-life of 6 hours,[11] resulting in an increase in PT.[5] This is often accompanied by non-specific clinical signs including respiratory distress, pale mucous membranes, anorexia, polyuria, polydipsia, and lethargy as animals become hypocoagulable, resulting in severe hemorrhage and anemia.[1,2,11] As the intoxication progresses over 48 hours, the activities of all the vitamin K–dependent coagulation factors decrease, which can induce severe and multi-site hemorrhages such as hematuria, hematemesis, hemothorax, or hemoabdomen.[1,2] Prolongation of the aPTT and PT is expected in these patients because the intrinsic and common pathway factors are deficient at this time. Therefore, it is critical for these patients to receive plasma or blood transfusions for immediate restoration of the depleted supply of important coagulation factors and proteins to provide rapid resolution of hemorrhage.[5,11] Although FFP and FWB are the 2 most commonly cited blood transfusion products used for ARI stabilization, stored plasma, or frozen plasma (frozen plasma stored for over 1 year), may also be used to replenish lost protein as well.[15,16] Although doses have been documented at 9 mL/kg for FFP[5] and 20 mL/kg for FWB,[17] clinical convention to normalize PT and aPTT in cases of ARI typically requires an IV dose of 10 to 20 mL/kg FFP. The patient should also be treated with oral vitamin $K_1$ supplements daily for at least 2 weeks with warfarin ingestion or at least 6 weeks with some of the newer anticoagulant rodenticides until the PT is within normal limits 2 to 3 days after the cessation of treatment.[1,5,12] The goal of vitamin $K_1$ supplementation is to replenish the vitamin $K_1$ supply to restore normal function of vitamin K–dependent coagulation factors. Long-term dosing (2.5-5 mg/kg/d)[15,17] is necessary to allow for full recycling of the dependent coagulation factors over the next few weeks until the PT and aPTT parameters are normal when measured 2 to 3 days after cessation of vitamin K and must be given as long as the toxin exerts its effects in the body at toxic concentrations.[2,5,11] This length of time will vary depending on the type and generation of anticoagulant ingested.[5]

This study focused on treating ARI and other vitamin K deficiencies with blood products by comparing the efficacy of CPP or FPP to correct different coagulation variables. When comparing the baseline values in CPP and FFP with the findings in Culler et al, there were some similarities between the factor and fibrinogen concentrations (Tables 1 and 2). Both studies analyzed their plasma at the Comparative Coagulation Lab at the Cornell University Animal Health Diagnostic Center. When comparing CPP data with the measurements from Culler et al, fibrinogen, FVII, and FIX had very similar results. FII and FX were more varied. The FFP data between the 2 studies varied more widely between all parameters measured. Although this was not statistically compared across these 2 studies, the clinical variability may have little impact on overall hemostasis in vivo.

Our adsorbed plasma mixing results indicated that there was a dose-dependent decrease in PT and PTT with both the addition of CPP and FFP, notably higher than the reported standard therapeutic dose of 1:5 to 2:5.[5] However, while these values indicated a statistical insignificance between FFP and CPP, there may be clinical significance due to the complexity of physiology with unpredictable reactions between different proteins or factors in an in vivo setting. We also found that increased doses of CPP or FFP resulted in increased FII and FIX concentrations although, even at the highest doses, the values remained below 50% of the normal reference interval. However, even partial correction of severe factor deficiencies after the transfusion of FFP or CPP may restore hemostasis in clinical practice, and clinical improvement is

routinely seen at dosages of 1:5 to 2:5. Therefore, these data provide the basis for a future trial to investigate CPP as an alternative to FFP to treat severe ARI requiring plasma products.

Unlike the other measured vitamin K–dependent coagulation factors, there was a difference between CPP and FFP when measuring changes in FVII concentrations. Addition of CPP to adsorbed plasma increased the activity of FVII significantly more than the addition of FFP ($P = 0.04$). Although the median of FVII activities in CPP was somewhat higher than in FFP, this difference at baseline was not statistically significant. FVII zymogen undergoes activation to its enzymatic form, FVIIa, under cold storage conditions.[18] This activation is mediated by the contact pathway, and it is possible that the slow thawing process to prepare CPP increased the unit potency of FVIIa in CPP such that increasing volumes of this product added to adsorbed plasma more rapidly trigger coagulation than equivalent volumes of FFP.[18]

Fibrinogen is an acute phase reactant protein that plays a role in coagulation as the precursor of fibrin and supporting platelet aggregation.[19] During major bleeding events such as with ARI, fibrinogen is the first factor to be consumed.[20,21] Therefore, increasing the concentration of fibrinogen with plasma product supplementation is essential for preventing further hemorrhage. Our study indicated that FFP contained a significantly higher concentration of fibrinogen and corrected the adsorbed plasma at a greater rate in comparison to CPP ($P = 0.005$). Fibrinogen is a large protein and, therefore, due to the centrifugation process utilized to produce cryoprecipitate plasma, a fibrinogen-poor supernatant is produced.[22]

FX activity was measured in the preliminary trials, but after addition of either CPP or FFP to adsorbed plasma, the activity of FX remained below 1% (data not shown). We did not further pursue the measurement of FX and did not measure residual barium concentrations in our depleted plasma, which is a limitation of this study. It is possible that the use of an artificially FX-depleted bovine plasma, rather than human-deficient plasma, was the cause of low FX recovery. The FX assay's configuration also differs from FII, FVII, and FIX, assays by the use of a more dilute plasma mixture and a snake venom activator in addition to thromboplastin.[23–26] Additionally, it is possible that lack of detectable factor X activity was due to interference or direct interaction of residual barium precipitate with the assay reactants, leading to delayed endpoint formation.

The addition of CPP or FFP in doses predicted to mimic standard clinical veterinary transfusions did not correct any of the coagulation times or coagulation factors levels back into normal canine reference intervals. Throughout this study, there were multiple freeze/thaw events to allow for the removal of barium sulfate, preservation of the specimen, and transportation for analysis. A recent study indicated that thawing plasma increased aPTT and decreased FVII and FX.[27] However, a separate study indicated that there were no statistically significant differences in the concentrations of all coagulation proteins between control FFP and FFP that has been thawed and refrozen.[28] Other studies have shown that in aged plasma at rest at room temperature, FV and FVIII actively degrade.[29] However, because there is a decreased concentration of FVIII in CPP, the results should not be affected significantly.[4] Because not all factors were measured, it is unknown how their presence or absence could have influenced fibrin formation and, therefore, PT and aPTT.

Our ex vivo factor depletion protocol differs mechanistically from the vitamin-dependent coagulopathy of clinical ARI, making this a considerable limitation of this in vitro study. Nevertheless, the vitamin K–dependent factor depletion allowed a preliminary comparison of treatment options for coagulopathy without the induction of ARI, which would place dogs at risk for hemorrhage. In order to extend the results of our study, future clinical trials are necessary to directly compare FFP and CPP in the target patient population.

The barium sulfate protocol removes vitamin K–dependent factors from plasma due to the factors' gamma carboxylated residues that are not present in the other coagulation factors, which are tightly bound to calcium. These residues are not present in non–vitamin K–dependent coagulation factors; therefore, vitamin K–dependent factors are selectively adsorbed when combined with insoluble salts.[30] Ideally, when measuring the baseline values, all coagulation factors should be measured to more thoroughly characterize the factor content of our protocol. However, due to financial constraints and our focus on the vitamin K–dependent factors, the other factors were not measured. Another potential limitation of this study was that the same adsorbed plasma was used as the base for all mixing studies. Although the baseline barium-depleted sample was the same for each trial, the comparison between CPP and FFP was isolated without imposing another variable. Ideally, this study could be repeated with multiple barium samples.

Despite these limitations, our results indicate that CPP may be a potential alternative to FFP in treating vitamin K–dependent factor deficiency, such as seen in ARI, when plasma transfusions are indicated. However, to the authors' knowledge, there are currently no published guidelines on the dose or rate of transfusion for the use of CPP in ARI, although the findings of Culler et al comparing the vitamin K–dependent factor levels of CPP to FFP suggest that CPP could potentially be used with a similar dosing strategy as conventionally used for FFP. In our study, the calculated "dose" of CPP or FFP utilized may not equate to the needs of individual animal in the clinic. It is always important to treat each patient individually, and the duration of exposure and dose of the ingested toxin may be highly variable. In clinical practice, patients need an individualized treatment plan titrated to effect based on the severity of intoxication and robust concentration of active coagulation factors in the donor plasma, which was not fully queried in this study. Until a confirmatory clinical trial is completed, the authors recommend 10 to 20 mL/kg CPP to treat canine anticoagulant rodenticide intoxication as an acceptable alternative to FFP.

Our results indicate that CPP may be a suitable alternative to FFP in treating vitamin K–dependent coagulopathy such as ARI. CPP partially corrected the coagulation times and concentration of vitamin K–dependent coagulation factors to the same degree as FFP. CPP may provide a potentially less expensive alternative to FFP for treatment of ARI.

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## ENDNOTES

* Sodium citrate (dehydrate) USP
† RC12BP Sorvall centrifuge, Thermo Fisher Scientific, Asheville, NC.
‡ Sahara-III Maxitherm, Sarstedt, Nümbrecht, Germany.
§ Adapted from Dr. Mehmood-ul-Hasan at the Care Diagnostics Centre Laboratory in Pakistan, mehmood196@hotmail.com; July 2018. Available at: http://haematologywatch.net/making-adsorbed-plasma.php.
** LIQUID POLIBAR PLUS barium sulfate suspension (105% w/v, 58% w/w), Bracco Diagnostics Inc., Milan, Italy.
†† Automated mechanical endpoint clot detection instrument, STACompact, Diagnostica Stago, Parsippany, NJ.
‡‡ Rabbit brain thromboplastin reagent, Thromboplastin LI, Helena Diagnostics, Beaumont, TX.
§§ Semi-automated mechanical endpoint clot detection instrument, STart4, Diagnostica Stago, Parsippany, NJ.
*** Human substrate deficient plasma factors II and VII, George King Diagnostics, Overland Park, KS.
††† Kaolin and rabbit brain thromboplastin activating reagent, Dade Actin, Siemens Healthcare Diagnostics, Marburg, Germany.
‡‡‡ Human factor IX deficient plasma, George King Diagnostics, Overland Park, KS.
§§§ Statistics software program, Stata version 14.2, StataCorp, College Station, TX.

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