

Effects of storage over a 36-month period on coagulation factors in a canine plasma product obtained by use of plasmapheresis

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OBJECTIVE

To evaluate stability of coagulation factors in canine plasma obtained by use of plasmapheresis and stored over a 36-month period.

SAMPLE

Canine plasma obtained by use of plasmapheresis acquired from a commercial blood bank.

PROCEDURES

Coagulation testing for fibrinogen concentration and activity of factors II, V, VII, VIII, and IX and von Willebrand factor was performed on canine plasma obtained by use of plasmapheresis. Samples were obtained for testing at 6-month intervals from plasma stored for up to 36 months.

RESULTS

A simple mixed linear regression model was created for each analysis. Median value for the fibrinogen concentration was > 150 mg/dL for all time points, except at 467, 650, and 1,015 days of storage. Median value for factor VIII was > 70% only at 650 days. Median value for factor V was > 50% through 650 days. Median value for factors VII and X was > 50% through 833 days, and median value for factors II and VII was > 50% through 1,015 days. Median value for von Willebrand factor was > 50% for the entire study (1,198 days). Median value for factor X was always < 50%.

CONCLUSIONS AND CLINICAL RELEVANCE

Coagulation factors degraded over time at variable rates, and all labile factors remained at > 50% activity for longer than 1 year. Plasma collected by plasmapheresis potentially offers prolonged life span of some clotting factors. Plasmapheresis is an acceptable form of canine plasma collection for transfusion purposes, and further studies should be performed to determine all of its benefits. (*Am J Vet Res* 2019;80:578–585)

Plasma is a blood product commonly used in human and veterinary medicine to prevent or arrest hemorrhage via replacement of coagulation factors. A 2010 veterinary incidence study¹ revealed that the reasons for plasma transfusion have changed substantially since 2000, and management of coagulopathies (with or without hemorrhage) is now the primary reason for administration of a plasma product.^{2,3} Investigators have concluded that there is no benefit for prophylactic plasma transfusions in humans who do not have active hemorrhage.^{4–7} However, there is a paucity of extensive studies in veterinary medicine⁸; therefore, plasma products remain the standard treat-

ment for domestic animals with possible or active hemorrhage attributable to or complicated by deficiencies of coagulation factors.^{1,3,9}

Traditionally, FFP is plasma that has been separated from whole blood and frozen within 8 hours after collection.^{3,10} Guidelines from Australia¹¹ and the United Kingdom⁵ specify that FFP must contain $\geq 70\%$ FVIII activity and > 140 mg of fibrinogen/dL. Canadian guidelines require $\geq 52\%$ FVIII activity.¹² The United States currently does not specify required activity.¹² Some human¹³ and veterinary¹⁴ studies have found that plasma can still be fresh-frozen if it is frozen within 24 hours after collection; such plasma is commonly labeled FP24. If used within 12 months after collection, FP24 contains all clotting factors, antithrombin, fibrinogen, albumin, and α -macroglobulins. After storage for ≥ 12 months, only certain clotting factors remain at acceptable activities, and the product is labeled FP.¹³

Plasmapheresis is an extracorporeal process that separates plasma and returns the remaining components of blood to the donor. This process has been found to be safe for human^{15,16} and equine¹⁷ blood

ABBREVIATIONS

FII	Factor II
FV	Factor V
FVII	Factor VII
FVIII	Factor VIII
FIX	Factor IX
FFP	Fresh-frozen plasma
FP	Frozen plasma
ICC	Intraclass correlation coefficient
vWF	von Willebrand factor
vWF:Ag	von Willebrand factor antigen

donors. Plasmapheresis allows for a greater yield of plasma from a donor during a single collection event than would be obtained with traditional methods and without any decrease in activity of coagulation factors for both humans¹⁸ and equids.¹⁷ To our knowledge, no studies involving plasmapheresis for transfusion to canids have been published.

The objective of the study reported here was to evaluate the relative clotting factor activity in canine plasma samples obtained by use of plasmapheresis and stored for a 36-month period. We hypothesized that FFP or FP obtained by use of plasmapheresis for a group of canine donors would have median FVIII activity > 70% after storage for 6 to 36 months.

Materials and Methods

Sample

Canine plasma samples (n = 70) were obtained by use of plasmapheresis. Ten adult (youngest was 3 years at first donation and oldest was 9 years at last donation) Greyhounds were used as blood donors. All dogs involved in the study were housed in an isolated facility of a commercial blood bank^a and did not have evidence of clinical diseases. Housing and care of the dogs met all mandatory regulations of the Australian Department of Agriculture.¹⁹ In addition, the facilities, donors, and products were approved by the Australian Pesticide and Veterinary Medicine Authority, and the donation procedures met the standards of and were monitored by the animal ethics committee within the Australian Department of Agriculture. Animals interacted frequently with caretakers for blood donation procedures and enrichment events to decrease stress. Blood donations typically were collected 9 times/y.

Plasmapheresis

All dogs were anesthetized with alfaxalone^b and inhalation anesthetics for blood collection. Each collection procedure was 1 to 1.5 hours in duration, and dogs were anesthetized and monitored throughout the entire period. A multicomponent apheresis collection system^c was used. Units were then stored in a freezer at -25°C at the blood bank. All freezers were human-grade equipment that were regularly calibrated with automated temperature monitoring systems.

Units of plasma were obtained from the donor dogs. These units initially were pooled and placed in Therapeutic Goods Administration–approved human-grade plasma transfer bags^d; plasma subsequently was packaged into smaller units for sale. Samples used in the study were 20- to 40-mL retention samples from the units, which were stored in the same plasma transfer bags^d and used immediately for quality control testing by the blood bank. These samples were also stored for 4 years after collection for testing in the event that adverse reactions were reported after administration of the units. Samples were stored in a freezer at -25°C at the blood bank.

Experimental procedures

Seven aliquots of FP were obtained for each of the 10 Greyhounds. The aliquots corresponded to the amount of time in storage. Samples for time 0 were acquired and evaluated as close as possible to the time at which they were obtained from each dog at the blood bank. The other 6 plasma samples were obtained from FP that had been obtained previously from each dog and stored frozen at -25°C; these samples represented storage for intervals of 6 months (ie, times 1 through 6 represented plasma samples that had been stored for 6, 12, 18, 24, 30, and 36 months, respectively). For example, plasma was obtained from dog 1 (time 0). The sample for time 1 was an aliquot from a unit of plasma that had been obtained from dog 1 six months previously and stored at -25°C. The sample for time 2 was an aliquot from another unit of plasma that had been obtained from dog 1 twelve months previously and stored at -25°C. The same procedure was used for samples at times 3 through 6.

Because of delays, time 0 was 102 days after plasma was obtained. The delay was attributable to selection of appropriate donors, company-regulated quality control assessments, timing of prescheduled donations, and shipment of samples. Samples 1 through 6 represented storage for 284, 467, 650, 833, 1,015, and 1,198 days, respectively.

The FP samples were shipped as a batch of 70 aliquots on dry ice to a coagulation laboratory^e for hemostatic protein testing. Temperature during shipment was monitored with a data logger.^f Mean temperature during shipment was -22.5°C (the highest temperature was -1.8°C [at the beginning of shipment], and the lowest temperature was -42°C). Time in shipment was approximately 7 days. Samples were stored at the coagulation laboratory at -25°C for 5 days before testing was performed.

Hemostatic testing

The samples were analyzed for fibrinogen concentration and activity of FII, FV, FVII, FVIII, FIX, FX, and vWF. Plasma samples were thawed at 37°C prior to assay. An automated coagulation instrument^g with mechanical endpoints was used to perform clotting time assays and the Clauss fibrinogen assay. Fibrinogen concentration was measured by use of commercial reagents^{h-j} and reaction conditions described elsewhere.²⁰ Fibrinogen concentration was measured with a standard curve derived from dilutions of single-use aliquots of a canine plasma standard prepared with plasma obtained from 20 healthy dogs. The fibrinogen concentration of the canine plasma standard was determined via a quantitative gravimetric method.²¹ The plasma vWF activity (determined as the vWF:Ag) was measured by use of an ELISA configured with monoclonal anti-canine vWF antibodies.²²

Coagulant activity for FVIII and FIX were measured by use of modified 1-stage activated partial thromboplastin time assays with a semiautomated clot detection instrument,^k human FVIII- and FIX-defi-

cient plasmas,¹ and a rabbit brain phospholipid^m with kaolin-activating reagent, as described elsewhere.²⁰ Coagulant activity assays for FII, FV, and FVII were performed by use of a modified 1-stage prothrombin time technique, rabbit brain thromboplastin reagent,ⁱ and human FII- and FVII-deficient plasmas,^h and coagulant activity of FX was determined by use of bovine-adsorbed artificially depleted plasma. Results of coagulant activity assays and vWF:Ag were reported as a percentage of the pooled canine plasma standard, which had an assigned value of 100% for factor activity and 100% for vWF:Ag.

Statistical analysis

Descriptive analyses (quartiles, interquartile [25th to 75th percentile] range, and median) were calculated for fibrinogen and each clotting factor. A Shapiro-Wilk test was performed to determine normality for all data sets.

A separate linear regression analysisⁿ was conducted for fibrinogen and each clotting factor. A simple mixed-linear regression model was used for each factor analysis (essentially time against outcome, with dog as a random effect) by use of the following equation: clotting factor activity = starting clotting activity at time 0 + time (ie, number of days) + random effect of dog. The random effect was included in the model for all samples, independent of the likelihood ratio or ICC. The hypothesis that the regression coefficient for time (slope of the line) was not significantly different from 0 (ie, the outcome [activity of coagulation factor] did not change over time) was tested by use of a *t* test. Significance was defined as $P < 0.05$.

Model validation was conducted by examining plots of residuals, examining pseudo r^2 values (values > 0.5 were consistent with a good fit), and assessing the assumption that a random effect was required in each model (by use of a likelihood ratio test). Validation by exclusion of data from the regression analysis and then predicting results for the excluded data was not possible because of the small sample size. The ICC was calculated for each factor and fibrinogen as follows: $ICC = \text{variance of the intercept} / (\text{variance of the intercept} + \text{variance of the residuals})$. Values > 0 , especially when they were close to 1, indicated that the data were clustered and an observation within a dog was more similar than observations among dogs. Values < 0 , especially when they were close to -1, indicated similarity among dogs rather than within a dog. The ICC was generally high, which indicated similarity of observations for each dog and indicated that a random effect was required to adjust the SE to minimize type 1 errors.

Results

Fibrinogen

Median fibrinogen concentration was > 150 mg/dL for 4 of 7 time points (**Table 1**). Fibrinogen concentration decreased over time. Although the r^2 was > 0.50 , the residuals were not normally distributed

(**Table 2**). Therefore, the model estimates should be interpreted with caution. Data variability was likely attributable to intradog variability (ie, day-to-day differences in clotting factor activity within each dog).

FII

Median activity of FII was $> 50\%$ for all time points, except for time 6 (**Table 1**). Activity of FII decreased over time; however, the residuals were not normally distributed, and the r^2 was < 0.50 (**Table 2**). Therefore, the model estimates should be interpreted with caution. Data variability was likely attributable to intradog variability.

FV

Median activity of FV was $> 50\%$ for the first 4 time points (**Table 1**). Activity of FV decreased over time. Although the r^2 was > 0.50 , the residuals were not normally distributed (**Table 2**). Therefore, the model estimates should be interpreted with caution. Data variability was likely attributable to intradog variability.

FVII

Median activity for FVII was $> 50\%$ for all time points, except for time 6 (**Table 1**). Activity of FVII decreased over time. Although the r^2 was > 0.50 , the residuals were not normally distributed (**Table 2**). Therefore, the model estimates should be interpreted with caution. Data variability was likely attributable to intradog variability.

FVIII

Median activity of FVIII was $> 70\%$ at only time 3 (**Table 1**). However, the median activity was $> 50\%$ for the first 4 time points. Activity of FVIII decreased over time. Data were normally distributed, and the model was a good fit (**Table 2**). Data variability was likely attributable to intradog variability.

FIX

Median activity for FIX was $> 50\%$ for the first 4 time points (**Table 1**). Activity of FIX decreased over time. Data were normally distributed, and the model was a good fit (**Table 2**). Although the r^2 was > 0.50 , the residuals were not normally distributed. Data variability was likely attributable to intradog variability.

FX

Median activity of FX was always $< 50\%$ (**Table 1**). Activity of FX decreased over time. Data were normally distributed, and the model was a good fit (**Table 2**). Although the r^2 was > 0.50 , the residuals were not normally distributed. Data variability was likely attributable to intradog variability.

vWF

Median activity of vWF was $> 50\%$ for all time points (**Table 1**). Activity of vWF decreased over time. Data were normally distributed, and the model was a good fit (**Table 2**). Although the r^2 was > 0.50 , the

Table 1—Median (interquartile [25th to 75th percentile] range) values for clotting factors in plasma samples obtained from each of 10 Greyhounds and stored frozen for up to 36 months.

Factor	Time						
	0	1	2	3	4	5	6
Fibrinogen (mg/dL)	176 (142–207)	232 (176–257)	132 (123–181)	121 (116–127)	150 (128–175)	135 (120–182)	169 (116–200)
FII (% activity)	64.5 (62.0–70.0)	68.0 (60.5–81.3)	66.0 (61.5–76.8)	58.0 (55.0–69.8)	50.5 (45.0–58.0)	50.5 (47.0–56.5)	47.0 (44.3–49.5)
FV (% activity)	76.5 (67.5–88.8)	66.5 (57.0–77.8)	54.0 (46.3–83.3)	62.5 (52.5–78.3)	34.5 (31.3–44.4)	12.5 (10.5–32.0)	11.5 (5.5–12.8)
FVII (% activity)	95.0 (77.3–118.2)	74.5 (68.5–99.8)	78.5 (59.0–88.8)	77.5 (62.8–110.5)	60.0 (50.8–84.0)	57.0 (49.8–83.0)	48.5 (43.5–67.0)
FVIII (% activity)	68.8 (63.8–76.9)	68.4 (63.2–77.8)	65.8 (61.4–70.9)	73.4 (62.6–85.8)	54.0 (41.6–65.9)	45.8 (40.2–53.8)	32.6 (23.4–38.7)
FIX (% activity)	63.6 (60.1–67.7)	64.2 (60.3–71.2)	64.8 (55.9–67.6)	64.5 (58.3–72.8)	51.7 (45.5–56.8)	41.0 (39.7–46.1)	34.5 (34.0–41.8)
FX (% activity)	45.7 (37.4–58.6)	47.2 (33.9–53.3)	40.3 (28.7–53.3)	44.9 (40.2–50.0)	18.9 (15.7–25.1)	13.5 (9.8–15.4)	9.3 (7.4–12.3)
vWF (% activity)	95.7 (81.4–109.5)	89.8 (76.0–99.7)	90.3 (86.3–93.8)	89.8 (87.7–105.4)	83.1 (72.9–99.3)	83.3 (61.0–87.0)	90.9 (79.5–96.4)

Plasma was obtained by use of plasmapheresis from each dog and stored frozen. Seven units of plasma were analyzed for each dog, representing a sample acquired and evaluated as close as possible to the time at which it was obtained from each dog (time 0) and samples that had previously been obtained and been frozen for 6 (time 1), 12 (time 2), 18 (time 3), 24 (time 4), 30 (time 5), and 36 (time 6) months. Because of delays, time 0 was 102 days after plasma was obtained; times 1 through 6 represented storage for 284, 467, 650, 833, 1,015, and 1,198 days, respectively.

Table 2—Results of linear regression analysis and model fit for all clotting factors tested.

Factor	Slope	SE	95% CI	W	Pseudo r^2	χ^2	ICC
Fibrinogen (mg/dL)	−0.03	0.010	0.01–0.05	0.93*	0.61	27.23*	0.57
FII (% activity)	−0.02	0.004	0.01–0.03	0.79*	0.48	1.35	0.19
FV (% activity)	−0.06	0.007	0.05–0.07	0.88*	0.65	13.27	0.25
FVII (% activity)	−0.04	0.007	0.03–0.05	0.96*	0.75	43.58*	0.67
FVIII (% activity)	−0.03	0.004	0.02–0.04	0.97	0.60	1.06	0.21
FIX (% activity)	−0.03	0.003	0.02–0.04	0.97	0.67	1.45	0.27
FX (% activity)	−0.04	0.004	0.03–0.05	0.97	0.67	6.37*	0.32
vWF (% activity)	−0.01	0.004	0–0.02	0.99	0.53	15.24*	0.43

The 95% confidence interval (95% CI) represents a value for the slope and was calculated as slope \pm (1.96*SE). The degree of normality is indicated as W; values > 0.97 are considered to reflect a normal distribution.

*Value is significant ($P < 0.05$).

residuals were not normally distributed. Data variability was likely attributable to intradog variability.

Discussion

Analysis of plasma samples for the study reported here revealed an overall decrease of clotting factors over a period of 102 to 1,198 days. Each factor had a variable decrease over this period, and only 1 factor, vWF, had a median value that remained within the acceptable range for the entire duration of the study. Previous studies have used a factor activity of 50% as an acceptable threshold to support hemostasis.¹⁴ It has been suggested that coagulation factor activity as low as 30% is sufficient for clinical or surgical hemostasis.^{12,23} Use of products that are more specifically tailored to the needs of a patient (ie, contain the minimum amount of a necessary clotting factor) would allow for more efficient use of relatively limited blood products in veterinary medicine.

Requirements for factor activity in human FFP differ among countries, with the most stringent regulations requiring $\geq 70\%$ FVIII activity and > 140 mg of fibrinogen.^{5,11} Median FVIII activity was > 70% at

time 3 and > 50% at all time points through time 4. The emphasis on FVIII activity by regulatory bodies likely stems from its unstable nature. It is widely accepted that FVIII is a labile coagulation factor and also seems to be the labile factor that degrades the most rapidly after collection.^{24,25} However, except for patients with hemophilia A (FVIII deficiency), the clinical necessity of providing a transfusion product with $\geq 70\%$ FVIII activity remains to be proven. Some studies^{12,26,27} of humans revealed increases in endogenous FVIII production in previously healthy individuals after stressful events (eg, surgery or hemorrhage). There have been several studies^{28–33} in which investigators have identified preoperative measurement of the fibrinogen concentration as a more accurate predictor of perioperative hemorrhage in humans. Another study¹² of humans focused on the usefulness of viscoelastic testing and thrombin generation for testing the quality of plasma units. Investigators of 1 study³⁴ used thromboelastography to determine that canine FP was still hemostatically active after storage for 5 years, despite significant decreases in FV and FVIII activities.

Activity of FVIII in the study reported here changed in a nonlinear manner, with a median of < 70% in samples at times 0, 1, and 2; > 70% at time 3; and then < 70% at times 4 through 6. The results of FVIII testing in the present study indicated that a major contribution to the differences in activity at each time point was day-to-day differences in clotting factor activity within each dog. Because we did not test degradation of activity within an individual unit of plasma, but instead tested plasma from 10 dogs stored for various amounts of time, this finding suggested that FVIII activity might be less dependent on storage time and more on variations within each dog at the time of blood collection. Numerous conditions in humans can affect clotting factor activity before blood collection and storage. Age, gender, ABO blood group,³⁵ altitude at which a person lives,³⁶ anthropometry,³⁷ blood pressure, cholesterol concentration, and oral contraceptive use³⁸⁻⁴⁰ have been found to affect clotting factor activity. Although no studies have been conducted to examine differences in coagulation factor activity related to variables such as age, body weight, body condition score, or activity level of dogs at the time of blood collection, it is possible these may have contributed to the variations that were detected in the present study. Additional studies to analyze the activity of FVIII at the time of blood collection and plasma harvest in dogs exposed to various conditions would help determine which, if any, of these variables affect FVIII activity.

One interesting finding in the present study was the universally low activity of FX in the samples tested. In this study, median FX activity was never > 50% (Table 1). Factor X is widely considered to be a stable factor and is required in treatment of hemorrhage resulting from warfarin toxicoses and vitamin K deficiencies. Activity of FX is not a specific consideration in the human guidelines for the labeling of a unit as fresh-frozen,¹² and the specific activity required for hemostasis is unknown.

Several explanations are possible for the low FX activity in the samples, including breed-related differences in FX activity, deterioration because of freeze-thaw cycles, and variations within or among dogs at the time of blood collection. One study¹⁴ showed that activity of FX is significantly lower in plasma obtained from Greyhounds than in plasma obtained from other breeds. In that study,¹⁴ a possible excess of citrate in the samples may have caused an *in vitro* decrease in FX activity. This was based on a study⁴¹ of humans in which investigators detected a decrease in FVIII activity in polycythemic patients caused by an increase in the citrate-to-plasma ratio and citrate binding of ionized calcium in the factor assays. However, this is unlikely to offer a relevant explanation for the low FX activity in the study reported here. First, in the aforementioned study,⁴¹ FX activity was not specifically evaluated; therefore, citrate interference of FX assays remains unknown. Second, FX activity in the present study was measured in plasma obtained by use

of plasmapheresis instead of whole blood collection into a citrate-containing bag. This removed breed-related polycythemia as a factor; therefore, an increase in the citrate-to-plasma ratio as a cause of an *in vitro* decrease in any clotting factor was unlikely. To the authors' knowledge, specific remarks about low FX activity in Greyhounds in blood collected for transfusion purposes were made in only 1 study¹⁴; thus, the tendency for that breed to have low FX activity remains unproven.

Second, freezing and thawing of samples can decrease FX activity. Investigators of 1 study⁴² tested canine FFP stored for 12 months and found a significant decrease in FX activity after storage. However, investigators of another study⁴³ determined that a freeze-thaw cycle had no deleterious effects on canine hemostatic protein activity. In the study reported here, FP was shipped directly to a laboratory, where it was thawed and tested with no refreezing process. Thus, freeze-thaw cycles were unlikely to have contributed to factor degradation. A data logger was used during shipment, and results confirmed that the temperature during shipment was maintained between -18° and -25°C.

Finally, similar to the discussion of variations in FVIII activity, it is possible that the low FX activity was attributable to intradog or interdog variability at the time plasma was obtained. Results of the present study indicated that similar to differences in FVIII activity, differences in FX activity at each time point also appeared to be more related to variability within each dog. Also similar to effects on FVIII activity, some dogs in the present study had good FX activity on the basis that 2 dogs had > 50% FX activity up to time 1, and 2 other dogs had > 50% FX activity through time 3 and 4. It also was possible that the plasmapheresis method of collection was responsible for the low FX activity. Further studies are needed to investigate these possibilities.

Fibrinogen is a protein produced by the liver, and it is converted to fibrin during the cascade of clotting events. Median fibrinogen concentrations remained within reference limits for all time points, except times 2, 3, and 5. This differed from results of other veterinary^{10,43,44} and human⁴⁵⁻⁴⁷ studies in which it was reported that fibrinogen remained within reference limits during storage under various conditions; however, fibrinogen concentrations were not evaluated over a prolonged period in any of those studies, which was in contrast to the present study. The model used for fibrinogen in the study reported here did not have a good fit; thus, it is difficult to interpret model estimates.

To the authors' knowledge, the plasma product evaluated in the study reported here is the only canine plasma product obtained by use of plasmapheresis that is currently available in the United States. Almost all of the human plasma for fractionation into derivatives is obtained via plasmapheresis.^{18,48} Although human FFP typically is obtained from whole

blood collections, it is possible to obtain it as a by-product of platelet or RBC apheresis,¹⁸ which therefore makes plasmapheresis a valuable tool for human transfusion medicine.

Veterinary research on plasmapheresis as a method of obtaining plasma for transfusion is limited to a few studies.^{17,49,50} Studies^{18,48,51,52} in human medicine suggest that in addition to allowing for a higher volume of plasma to be obtained from a donor, plasma obtained by use of plasmapheresis also contains higher activities of FV, FVIII, FIX, and FXI. However, results of the study reported here suggested that some factors (eg, FX) may have poor stability in canine plasma obtained via plasmapheresis. The authors are not aware of any human studies that have described FX activity in plasma obtained by use of plasmapheresis.

An additional potential benefit of plasmapheresis as a collection method is an improved life span of some clotting factors. Studies^{53,54} of humans have revealed that when collection involves whole blood, proteolytic enzymes released from platelets, erythrocytes, and leukocytes will degrade or destabilize clotting proteins. Because these cells are removed via filtration during the collection process, this effect is detected less frequently with plasmapheresis.⁴⁸ This could explain the longevity for activity of certain factors in the units tested in the present study.

Finally, a clear benefit of plasmapheresis is the ability to safely obtain a large volume of plasma at 1 time, compared with the amount of plasma obtained for a whole blood donation.^{18,51} Typically, the amount of plasma obtained for a whole blood donation is approximately 220 mL, whereas plasmapheresis can yield from 450 to 880 mL of plasma.^{15,51} A larger volume of plasma from 1 donor source would spare a recipient the necessity of a plasma transfusion with plasma from multiple donors. Theoretically, this could decrease the risk of transfusion reactions in patients that require large volumes of plasma for resuscitation. Further studies on plasma obtained by use of plasmapheresis as an alternative method for collection of blood components could provide a novel option for transfusion treatments in veterinary medicine.

The present study had some limitations. One limitation was that clotting factors and fibrinogen were not quantified at the time of blood collection for any plasma unit (including the time 0 control units). Thus, the initial factor activity for each unit was not known, which made it impossible to determine the variability of factor activity within each dog at various collection times. Future studies that account for the initial activity of clotting factors would allow investigators to develop an estimated timeline of degradation.

An additional limitation was the use of plasma units obtained at different time points, rather than tracking the degradation of clotting factor activity within the same unit over time. This likely introduced variability that contributed to the nonlinear regression of factor activity. The study reported here was

intended to determine whether randomly selected units of plasma obtained from a group of donors and stored for various amounts of time would have clotting factor activities adequate for clinical use. Future studies that span the proposed 3-year shelf life and monitor factor activity in individual units over that period could better characterize degradation of factor activity over time.

The plasma product used in the study reported here was obtained from Greyhounds that were part of a blood donor colony. Greyhounds reportedly have bleeding tendencies. In 1 report,⁵⁵ survey results indicated that 10% to 15% of Greyhounds had hemorrhage 1 to 4 days after routine procedures, and many of the patients even required a transfusion. This hemorrhage was despite the fact the Greyhounds had results of coagulation testing that were within reference limits.⁵⁵ Despite the reported *in vivo* tendency for hemorrhage in Greyhounds, there has not been a link between bleeding tendency and clotting factor deficiencies in that species.⁵⁶ Regardless, results of the study reported here should be applied only to plasma obtained from Greyhounds. Further studies involving dogs other than Greyhounds are warranted to elucidate possible differences in factor activities among breeds for plasma obtained via plasmapheresis.

The labile FVIII had a median factor activity $\geq 70\%$ at only time 3 and $\geq 50\%$ at times 0 through 4. Further studies are required to determine the clinical necessity of transfusing a product that has such high factor FVIII activity. Median factor activity of FV was $\geq 50\%$ through time 3. To our knowledge, this was the first study conducted to examine clotting factor activity in canine plasma obtained by use of plasmapheresis. Further investigation into use of this method is warranted because plasmapheresis could provide a valuable alternative to current methods used to obtain canine plasma.

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Footnotes

- a. Plasvacc, Kalbar, QLD, Australia.
- b. Jurox Pty Ltd, Rutherford, NSW, Australia.
- c. Haemonetics, Braintree, Mass.
- d. Terumo Medical, Tokyo, Japan.
- e. Comparative Coagulation Laboratory, Animal Health Diagnostic Center, Cornell University, Ithaca, NY.
- f. Escort iMiniPlus PDF temperature logger, Cryopak, Edison, NJ.
- g. STA compact, Diagnostica Stago, Parsippany, NJ.
- h. Dade Actin FS, Siemens Health Diagnostics, Marburg, Germany.
- i. Thromboplastin LI, Helena Diagnostics, Beaumont, Tex.
- j. Fibrinogen, Diagnostica Stago, Parsippany, NJ.

- k. ST4, Diagnostica Stago, Parsippany, NJ.
- l. Factor deficient plasmas, George King Bio-Medical, Overland Park, Kan.
- m. Dade actin, Siemens Health Diagnostics, Marburg, Germany.
- n. R Project, version 3.3.2, nlme package, R Foundation for Statistical Computing, Vienna, Austria.

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