Original Article

Hematological, biochemical and microbiological evaluation of feline whole blood units collected using an open system and stored for 35 days

E. Spada [a,*], R. Perego [a], L. Baggiani [a], P.A. Martino [b], D. Proverbio [a]

[a] *Veterinary Transfusion Research Laboratory (REVLab), Department of Veterinary Medicine (DIMEVET), University of Milan, via G. Celoria, 10 – 20133, Milan, Italy*
[b] *Laboratory of Microbiology and Immunology, Department of Veterinary Medicine (DIMEVET), University of Milan, via G. Celoria, 10 – 20133, Milan, Italy*

Corresponding author: Tel: +39 025 031 8188.
E-mail address: eva.spada@unimi.it (E. Spada).

Abstract

Despite the increasing availability of feline blood, which is collected and stored for transfusion purposes, few studies have assessed the effect of storage on feline whole blood (WB) units. The purpose of this study was to investigate selected hematologic and biochemical changes during storage of feline WB units and to determine when they occurred. Data from a quality control program for WB units was used in this study. Twelve feline WB units, collected using an open system, were sampled every 7 days from the point of collection to the end of storage at 35 days (D0, D7, D14, D21, D28, and D35). Measurements at each time point were: (1) hematologic parameters; (2) percentage hemolysis; (3) morphologic index scored at 0 - 3, based on echinocyte transformation of the erythrocytes; and (4) selected biochemical parameters. Aerobic and anaerobic culture was performed at D0 and D35. Results were compared statistically to D0 (statistical significance set at <0.01).

Storage did not result in statistically significant changes in measured hematological parameters. There were statistically significant increases in percentage hemolysis and morphologic index, starting from D21 (P=0.000 and P=0.004, respectively). Glucose decreased significantly from D21 (P≤0.003); potassium increased significantly from D7 (P≤0.003); and sodium increased significantly, starting from D28 (P=0.009). Bacteria were not isolated. Blood in feline WB units collected using an open system underwent some significant storage changes that were time-dependent. As these changes could affect the quality and the utility of stored WB used in feline transfusion medicine, further study is required to determine their clinical importance.

41 *Keywords*: Feline; Hemolysis; RBC morphology; Storage lesion; Whole blood unit

42

Introduction

Despite the growing interest in veterinary transfusion medicine, little is known about feline transfusion medicine, and most information is still extrapolated from human and canine transfusion medicine.

Though easily adaptable for dogs, commercial blood collection sets for human use are not usable for feline blood collection because their blood volume is too small for standard 450 mL human closed-collection systems. For these reasons and because feline-specific closed blood collection systems have limited availability, open or semi-closed collection systems (in which anticoagulant must still be added through an injection port before collection) are most commonly used for feline blood donation (Blasi Brugué et al., 2018; Heinz et al., 2016; Spada et al., 2018, 2017; Weingart et al., 2004). During blood donation, feline blood is usually collected into a syringe through a butterfly catheter and, if collected for storage, transferred aseptically to a 50-150 mL transfer-pack container and sealed to prevent bacterial contamination (Lucas et al., 2004). The small volume of blood collected from each cat (approximately 50-60 mL) poses challenges for separation of the whole blood (WB) unit into components, such as packed red blood cells (PRBC) and plasma units. Given the difficulties in obtaining specific blood components, WB is most commonly used in feline transfusion medicine.

Commentato [AL1]: Multiple citations should be arranged chronologically, starting with the oldest citation, not alphabetically. Please correct here and throughout the manuscript.

There is a scarcity of published information on feline WB storage time (Bücheler and Cotter, 1994; Crestani et al., 2018) and although legal standards for storage do not exist, feline WB units are usually stored for 21 (Lucas et al., 2004) to 35 days (Bücheler and Cotter, 1994; Crestani et al., 2018).

The Food and Drug Administration (FDA), which regulates the collection and storage of human blood products used for transfusion in the USA, relies primarily on two measures of efficacy and safety: 24-h recovery and survival >75% of radiochromium-labelled red blood cells (RBCs), and hemolysis <1% at the end of the approved storage period (FDA, 1985). No hemolysis limit has been officially adopted in veterinary transfusion medicine, but the <1% hemolysis limit imposed in human medicine has been frequently used for blood unit evaluation in veterinary studies (Blasi Brugué et al., 2018; Crestani et al., 2018; Ferreira et al., 2018; Price et al., 1988).

Changes in the biochemical and biological properties of RBCs during storage are termed storage lesions. These include specific changes in RBC morphology, from deformable biconcave discs to poorly deformable echinocytes, and ultimately, non-deformable spheroechinocytes, and biochemical changes derived from RBC metabolism. Morphological changes reduce the survival time of RBCs in the recipient and affect the capacity of RBCs to distribute oxygen and remove carbon dioxide from tissues (Berezina et al., 2002; Blasi et al., 2012; Obrador et al., 2015). Additionally, some biochemical changes could potentially harm the recipient. Storage changes such as echinocyte transformation, increased hemolysis, decreased glucose, and increased potassium have been documented in feline WB units after 35 days storage (Crestani et al., 2018; Spada et al., 2018), but the chronology of these changes during storage have not been investigated.

Given the lack of information on storage lesions in feline WB units collected using an open system, the aims of this study were: (1) to investigate selected hematologic and biochemical changes that occur during feline WB unit storage; and (2) to determine when the changes occur with respect to time since collection. We hypothesized that during blood storage, there would be a progressive increase in hemolysis, echinocyte shape transformation and biochemical changes consistent with RBC metabolism in a closed system e.g. decreased glucose content and increased potassium.

Materials and methods

Donor population and blood collection

The study was performed on non-leukoreduced feline WB units (volume, 60 mL) from the Veterinary Transfusion Research Laboratory (REVLab), University of Milan, Milan, Italy, in 2018. Suitable feline blood donors donated blood under general anesthesia after informed owner consent, following the guidelines on veterinary transfusion from the Italian Health Minister[1] and as previously described (Spada et al., 2015). Briefly, blood was collected with a ratio of citrate phosphate dextrose adenine (CPDA) anticoagulant-preservative solution:blood of 1:7, using an open system consisting of three 20 mL syringes.

Blood was transferred to a 150 mL empty transfer bag (TERUFLEX Transfer Bag, TERUMO EUROPE) using a sterile bag spike (Combifix Adapter; B Braun Vet Care) placed aseptically in each bag. Blood units were stored in a controlled-temperature dedicated blood

[1] See: Italian Ministry of Health, 2016. *Linea guida relativa all'esercizio delle attività riguardanti la medicina trasfusionale in campo veterinario*. http://www.trovanorme.salute.gov.it/norme/renderPdf.spring?seriegu=SG&datagu=01/02/2016&redaz=16A00611&artp=1&art=1&subart=1&subart1=10&vers=1&prog=001 (accessed 13 October 2019)

bank refrigerator (EMOTECA 250, Fiocchetti), with a continuous temperature record and alarm, and the temperature was consistently maintained at 4±2 °C. Units were stored vertically and manually mixed gently and inverted every 48 h during the entire storage period to maximize cell exposure to the preservative solution.

Before sampling, feline WB units were gently mixed by inversion for 1 min, then a 1.5 - 5 mL aliquot was aseptically collected using the sterile bag spike. Blood samples were collected from the units at D0 (the day of blood collection) and every 7 days until 35 days of storage (D7, D14, D21, D28, and D35), i.e. the date of final storage/expiration of the WB units (Bücheler and Cotter, 1994).

After collection, samples were transported to an on-site clinical veterinary transfusion laboratory and all analyses were performed within 1 h by a single clinical pathologist who was not masked to storage time. Blood samples (1.5 mL) from all sampling days, were placed in an Eppendorf tube for hematologic evaluation and for morphologic evaluation of smears. The tube was centrifuged to obtain plasma for estimation of hemolysis, and measurement of glucose, sodium and potassium. At D0 and D35, a further 3.5 mL blood was kept in the original sampling syringe and submitted for microbiologic analysis.

All the analyses were performed as part of a regular quality control program for feline blood unit production at REVLab. For this study, one feline WB unit was analyzed each month for a total of 12 months.

The study was conducted using a protocol approved by the University of Milan Animal Welfare Bioethical Committee (OPBA_26_2018_permission). Written owner consent for blood collection, use of blood samples, and use of data for scientific purposes, was routinely obtained during feline consultations and prior to blood donation.

Commentato [AL2]: Please provide approval date in the following format - 15 March 2018

Hematological parameters

The following hematological parameters were assessed using an automated multiparameter hematology analyzer with software for animal samples (Cell-Dyn 3500 analyzer, Abbott Diagnostics Europe): RBC count, hemoglobin (Hb), hematocrit (HCT), mean cell volume (MCV), mean cell Hb (MCH), mean cell Hb concentration (MCHC), and RBC distribution width (RDW).

Erythrocyte morphology and morphological index

Erythrocyte morphology was assessed using May Grunwald-Giemsa-stained (MGG Quick Stain, Bio-Optica) blood smears using light microscopy. Normal erythrocytes were scored 0, and echinocytes were scored from +1 to +3 as follows: echinocyte I (score +1), irregularly contoured discocyte with up to five protrusions; echinocyte II (score +2), flat cell with multiple spicules; and echinocyte III (score +3), ovoid or spherical erythrocyte with multiple spicules. For each sample, 200 RBCs were scored and the morphological index (MI) was calculated as the sum of scores/200 (Ergül Ekiz et al., 2012; Sollberger et al., 2002).

Hemolysis

Following measurement of hematologic parameters and blood smear examination, blood samples were centrifuged at 3500 *g* for 10 min; free Hb was measured in the plasma using an automatic analyzer (Cell-Dyn 3500 analyzer, Abbott Diagnostics). Hemolysis was reported as a percentage, calculated using HCT and the concentrations of total and plasma Hb according to the following equation (Blasi Brugué et al., 2018; Crestani et al., 2018; Niinistö et al., 2008; Sowemimo-Coker, 2002; Wardrop et al., 1997):

Hemolysis (%) = [(100 – HCT (%)] x [supernatant Hb (g/dL) / total Hb (g/dL)].

The Hb detection range of the Cell-Dyn 3500 based on linearity studies[2] was 0–24 g/dL.

Commentato [AL3]: Please use SI units (mmol/L) for hemoglobin here and throughout the manuscript, including tables.

Commentato [AL4]: Please check that the URL below is correct - the one you had provided was no longer active.

Biochemical parameters

Selected serum biochemical parameters, including glucose, sodium, and potassium concentrations, were evaluated in CPDA plasma samples from feline WB units. Glucose was measured spectrophotometrically using an automated analyzer (COBAS MIRA Classic; Roche Diagnostics). Sodium and potassium concentrations were determined using a flame photometer (IL 943; Instrumentation Laboratory).

Microbiologic analysis

To evaluate possible bacterial contamination, microbiologic analysis was performed on feline WB samples at D0 and D35. Duplicate blood samples were seeded aseptically in tryptic soy broth (Oxoid) at a ratio of 1:10. Then tubes were incubated at 37 °C for 24 h under aerobic and anaerobic conditions (BBL GasPak Plus System; Becton Dickinson). After incubation, if the broth-culture was clear (negative; turbidity evaluation based on comparison with a negative

[2] See: Abbott Cell-Dyn 3500 System, Operator's Manual
https://resources.psmile.org › abbott › cell-dyn › at_download › file
(accessed 13 October 2019)

control uninoculated tube containing tryptic soy broth), it was incubated again under the same conditions for a further 24 h (repeated for a total of up to 72 h). If a positive culture was detected (turbid sample), 100 µL of the broth was plated onto blood agar plates (Oxoid) and incubated at 37 °C for 24-48 h under aerobic or anaerobic conditions (depending on the incubation conditions previously used for the positive sample). Colonies were identified by macroscopic and microscopic evaluation (e.g. Gram stain), biochemical tests and use of selective media (e.g. MacConkey agar for *Enterobacteriaceae*, mannitol salt agar for *Staphylococcaceae*; Markey et al., 2013).

Statistical analysis

Data were analyzed statistically using statistical software (MedCalc version 16.4.3). Data distribution was assessed using the Shapiro-Wilk test. Comparison of differences between D0 and all other time points (D7, D14, D21, D28 and D35) was performed using paired Student's *t*-tests or Wilcoxon signed-rank tests for paired samples, for normally and non-normally distributed data, respectively. Statistical significance was set at $P<0.01$. Correlation between storage time and degree of hemolysis, and between MI and degree of hemolysis, was assessed by calculating Pearson correlation coefficients (r).

Results

Twelve feline WB units were analyzed at D0 and D35 for all parameters. For technical reasons, 7/12 units were analyzed at D7 and D14, and 9/12 units were analyzed at D21 and D28.

Hematologic parameters

199 Results of hematologic evaluation of the feline WB units are reported in Table 1.
200 Hematologic parameters did not change from D0 for any storage time point.
201
202 *Morphologic index*
203 Results of morphological evaluation are reported in Table 2. Erythrocyte MI increased
204 significantly compared to D0 from D21 onwards (P=0.003).
205
206 *Hemolysis*
207 Mean and median percentage hemolysis are reported in Table 3. Considering 1%
208 hemolysis as the maximum acceptable value for quality blood units, as recommended in human
209 transfusion medicine by the FDA, all feline WB units had hemolysis < 1% at D0 (Fig. 1). There
210 was a significant increase in hemolysis compared to D0 from D21 onwards (P<0.000). Storage
211 time and hemolysis were positively correlated (r = 0.73; P<0.001, 95% confidence intervals
212 0.58, 0.83; Fig. 1). The Pearson correlation coefficient between MI and hemolysis was 0.65 (P
213 <0.001, 95% confidence intervals 0.46, 0.78; Fig 2).
214
215 *Biochemical parameters*
216 Biochemical parameters are reported in Table 4. Glucose decreased significantly from
217 D21 onwards (P=0.003). Sodium concentrations increased significantly compared to D0 only at
218 D28 (P=0.009). Significant increases in serum potassium concentrations occurred from D7
219 (P=0.001).
220
221 *Microbiologic analysis*

Commentato [AL5]: Please express these values as a number greater than zero - make changes as appropriate throughout the manuscript and tables.

No changes in color (brownish or purplish discoloration) were observed in any of the stored feline WB units. No aerobic and anaerobic bacteria were cultured from any of the samples submitted at D0 and D35.

Discussion

Changes in hematologic and biochemical parameters during storage of blood units or components are important in determining maximum storage time before blood transfusion. Hematologic, morphologic and biochemical parameters were monitored for 35 days in 12 feline WB units in this study. After storage for 21 days, the percentage hemolysis and echinocyte transformation of normal erythrocytes in feline units increased significantly compared to values at collection (D0).

In agreement with recent studies performed in feline WB units collected with an open (Spada et al., 2018) and a closed system (Crestani et al., 2018), no statistically significant changes in hematologic parameters were demonstrated during storage in this study.

There is some inevitable damage to some RBCs during the collection, processing and storage of blood for transfusion purposes (Acker et al., 2012; Sowemimo-Coker, 2002). Hemolysis must be minimized to maintain the quality of the transfusion product, as high concentrations of Hb have toxic effects on myocardial, renal, vascular and central nervous system tissues (Buehler and D'Agnillo, 2010). In human medicine, accidental transfusion of hemolyzed blood has resulted in hemoglobinemia and hemoglobinuria (Sandler et al., 1976;

Sloan et al., 2009), and in consumptive coagulopathy (Sloan et al., 2009). Consumptive coagulopathy was the suspected cause of death in a canine hemorrhagic shock model in which dogs received 2 mL/kg of hemolyzed (frozen and thawed) autologous blood (Hardaway et al., 1979). Inappropriate storage conditions may have resulted in increased hemolysis in blood units transfused to four dogs that went on to develop acute, life-threatening, transfusion reactions (Patterson et al., 2011). These studies underline the potential harmful effects of free Hb in hemolyzed stored canine blood. A recent study (Blasi Brugué et al., 2018) reported mean percentage hemolysis of 0.07% in feline PRBC units when stored for <24 h. However, when these units were preserved for up to 28 days, 13.8% of units exceeded the 1% FDA limit. Another recent study found that eight feline WB units collected using a closed system had mean hemolysis <1% after storage for 35 days (Crestani et al., 2018). In our study, mean percentage hemolysis was lower than the FDA limit for only the first 21 days of storage, i.e. 2 weeks shorter duration of storage than the study by Crestani et al. (2018). The difference in percentage hemolysis at 28 days of storage was not considered to be related to the different systems used for blood collection, as hemolysis at point of collection (D0) was <1% for both collection systems. In addition, a recent study on stored canine PRBC units (Ferreira et al., 2018) reported no correlation between pre- and post-storage hemolysis, or any effects due to disturbances in the collection process.

Different collection systems could affect the amount of hemolysis at the time of collection. A previous study (Crestani et al., 2018) used a feline-specific closed system with a special self-cleaning valve to sample blood from the unit to be analyzed. We hypothesize that this valve reduced the potential for shear stress derived from sample collection. Additionally, as

blood in this closed system went directly from the collection syringe to the blood bag, with no RBC stripping in the sample tube, shear stress and hemolysis might have been reduced (Sowemimo-Coker, 2002). This hypothesis is supported by the low mean percentage hemolysis at collection in a previous study (Crestani et al., 2018), which was substantially less (i.e. 0.11%) than the hemolysis at D0 in our study. This emphasizes the importance of species-specific equipment to facilitate blood donation in small animals, such as cats, to improve the quality of blood units.

The results of our study emphasize the need to implement quality control programs in veterinary blood banks so that hemolyzed WB units can be identified. Thus, despite the generally accepted shelf-life of 35 days for feline WB units, one should be aware that any WB unit stored for >3 weeks should be evaluated for hemolysis as an indicator of its viability. However, our results do not support the practice of disposing of units after >3 weeks, as almost half of the feline WB units we studied had acceptable hemolysis values for up to 5 weeks of storage. Additionally, the effects of transfusing blood with >1% hemolysis into feline blood recipients have not been investigated.

RBCs undergo progressive shape changes during storage, from deformable discs to more rigid echinocytes. Echinocytes are less deformable than discocytes and at high shear rates, their cell spicules become entangled, increasing blood viscosity (Sollberger et al., 2002). This change is initially reversible, but once spheroechinocytes are formed, these changes are permanent (Berezina et al., 2002; Obrador et al., 2015). Morphological assessment of RBCs in preserved blood units, such as those performed in our study, might therefore provide important information

on the quality of blood units. Statistically significant alterations of RBC shape, as shown by progressive increase in MI, started during the second week of storage. In accordance with the results of previous studies (Crestani et al., 2018; Spada et al., 2018), in which there were statistically significant increases in echinocyte numbers between D0 and D35 of storage, the changes in RBC shape in our study were accompanied by progressive increases in MI and hemolysis. However, in vivo studies are needed to understand whether morphological changes detected during feline WB storage could be reversed after transfusion and to determine the clinical consequences of transfusing such altered RBC into the recipient.

Changes in selected biochemical parameters evaluated in this study were consistent with RBC metabolism in a closed system, as previously documented in feline WB and PRBC units (Crestani et al., 2018; Heinz et al., 2016). Although there were statistically significant changes in biochemical parameters from D0 in this study, the overall magnitude of these changes was unlikely to be clinically relevant and are unlikely to cause clinically significant electrolyte disturbances in recipient cats. In human transfusion medicine, potentially fatal complications can occur after rapid infusion of stored units due to excessive serum potassium concentrations. Feline RBC membranes lack sodium–potassium-ATPase pump activity (Chan et al., 1964), suggesting limited capacity for potassium accumulation in stored feline WB units. In our study, although serum potassium concentration increased during early storage, the median concentration at D35 was low and within the feline potassium reference interval. In addition, as feline WB units are diluted in the total feline blood volume after transfusion, this potassium change should not harm the recipient; this may be true for most biochemical changes during the blood storage.

Serum glucose concentration decreased during storage due to consumption by RBCs. However, due to the dextrose content of CPDA-1, glucose concentrations remain persistently high at the end of storage, as previously shown (Crestani et al., 2018). Care should be taken in hyperglycemic cats that receive large transfusions of fresh or stored WB units.

This study has some limitations. Hemoglobin concentration used for calculation of percentage hemolysis was measured using an automated analyzer rather than accepted reference methods (Drabkin's cyanmethemoglobin and the Harboe spectrophotometric method). Based on linearity studies, the automated analyzer used in this study has a Hb detection range of 0–24 g/dL. However, linearity studies cannot reliably determine if the analyzer can distinguish very low Hb concentration, e.g., 0.1 vs. 0.0 g/dL. Because of this, it is difficult to determine analyzer accuracy at very low Hb concentrations. Additionally, hemolysis measurements could have been affected by the method of analysis. The measurement of hematocrit can also be a source of bias, since hematocrit is a calculated value. Substantial bias has been reported when automated analyzers were used to estimate hemolysis, and some analyzers over-estimate total Hb concentration (Acker et al., 2012). Since we used an automated analyzer to measure Hb, percentage hemolysis could have been overestimated in our study.

Just one clinical pathologist evaluated the morphologic changes and she was not masked to sample storage time. This could have reduced objectivity when evaluating morphological change, but should not have affected the final assessment of erythrocyte changes during storage.

The number of samples was relatively small, and at some time points only a limited

number of blood units was analyzed. This may have reduced our ability to detect differences between samples by reducing statistical power. The limited number of total feline WB units analyzed in this study represented the number of units used for quality control in a veterinary blood bank over a year. Feline blood units are a limited resource, as feline blood donors were limited (in comparison with canine blood donors); therefore, the number of units analyzed in this study reflects the limitations encountered in feline transfusion medicine.

Conclusions

Feline WB units underwent some changes during storage. Biochemical changes, such as increased serum sodium, potassium and decreased glucose concentrations, are unlikely to be clinically relevant in recipients, as the changes were minor and WB units are diluted in the circulation of the recipient after transfusion. More important changes included increased hemolysis and echinocyte transformation in stored RBCs. These changes should be clinically evaluated in in vivo studies to better understand their effects on the quality and safety of feline WB units after weeks of storage.

Conflict of interest statement

The authors have no financial and personal relationships with people or organizations that could have inappropriately influenced his work.

Acknowledgements

This study was supported, in part, by *Piano di Sostegno alla Ricerca 2017, Linea 2*, University of Milan, Milan, Italy and by FFABR *(finanziamento individuale per le attività di*

base di ricerca). Part of the results of this study was presented as poster at 24th International Veterinary Emergency and Critical Care (IVECCS) Symposium, New Orleans, LA, USA, 14-18 September, 2018.

References

Acker, J.P., Croteau, I.M., Yi, Q.-L., 2012. An analysis of the bias in red blood cell hemolysis measurement using several analytical approaches. Clinica Chimica Acta 413, 1746-1752.

Berezina, T.L., Zaets, S.B., Morgan, C., Spillert, C.R., Kamiyama, M., Spolarics, Z., Deitch, E.A., Machiedo, G.W., 2002. Influence of storage on red blood cell rheological properties. The Journal of Surgical Research 102, 6-12.

Blasi, B., D'Alessandro, A., Ramundo, N., Zolla, L., 2012. Red blood cell storage and cell morphology. Transfusion Medicine 22, 90-96.

Blasi Brugué, C., Ferreira, R.R.F., Mesa Sanchez, I., Graça, R.M.C., Cardoso, I.M., De Matos, A.J.F., Ruiz De Gopegui, R., 2018. In vitro quality control analysis after processing and during storage of feline packed red blood cells units. BMC Veterinary Research 14, 141.

Bücheler, J., Cotter, S.M., 1994. Storage of feline and canine whole blood in CPDA-1 and determination of the posttransfusion viability. In: Proceedings of the 12th Annual ACVIM Forum. San Francisco, CA, USA.

Buehler, P.W., D'Agnillo, F., 2010. Toxicological consequences of extracellular hemoglobin: biochemical and physiological perspectives. Antioxidants and Redox Signaling 12, 275-291.

Chan, P., Calabrese, V., Theil, L., 1964. Species differences in the effect of sodium and potassium ions on the ATPase of erythrocyte membranes. Biochimica et Biophysica Acta 79, 424-426.

Crestani, C., Vascellari, M., Stefani, A., Carminato, A., Cro, A., Bozzato, E., Mutinelli, F., 2018. In vitro assessment of quality of citrate-phosphate-dextrose- adenine-1 preserved feline blood collected by a commercial closed system. Journal of Veterinary Internal Medicine 32, 1051-1059.

Ergül Ekiz, E., Arslan, M., Akyazi, Í., Eraslan Uygur, E., Inal Gültekin, G., Özcan, M., 2012. The effects of prestorage leukoreduction and storage duration on the in vitro quality of canine packed red blood cells. Turkish Journal of Veterinary and Animal Sciences 36, 711-717.

Ferreira, R.R.F., Graça, R.M.C., Cardoso, I.M., Gopegui, R.R., de Matos, A.J.F., 2018. In vitro hemolysis of stored units of canine packed red blood cells. Journal of Veterinary Emergency and Critical Care 28, 512-517.

Food and Drug Administration, 1985. Workshop on red cells stored in additive solution systems. April 25th 1985, Bethesda, MD, USA.

Hardaway, R.M., Dumke, R., Gee, T., Meyers, T., Joyner, J., Graf, J., Lee, D., Revels, J., 1979. The danger of hemolysis in shock. Annals of Surgery 189, 373-376.

Heinz, J.A., Pashmakova, M.B., Wilson, C.R., Johnson, M.C., Minnard, H.M., Bishop, M.A., Barr, J.W., 2016. Biochemical evaluation of the effects of storage on feline erythrocytes. Journal of Small Animal Practice 57, 637-643.

Lucas, R.L., Lentz, K.D., Hale, A.S., 2004. Collection and preparation of blood products. Clinical Techniques in Small Animal Practice 19, 55-62.

Markey, B.K., Leonard, F.C., Archambault, M., Cullinane, A., Maguire, D., 2013. Bacterial pathogens: Microscopy, culture and identification. In: Clinical Veterinary Microbiology, Second Edn. Mosby Elsevier, Missouri, USA, pp. 9-47

Niinistö, K., Raekallio, M., Sankari, S., 2008. Storage of equine red blood cells as a concentrate. The Veterinary Journal 176, 227-231.

Obrador, R., Musulin, S., Hansen, B., 2015. Red blood cell storage lesion. Journal of Veterinary Emergency and Critical Care 25, 187-199.

Patterson, J., Rousseau, A., Kessler, R.J., Giger, U., 2011. In vitro lysis and acute transfusion reactions with hemolysis caused by inappropriate storage of canine red blood cell products. Journal of Veterinary Internal Medicine 25, 927-933.

Price, G.S., Armstrong, P.J., McLeod, D.A., Babineau, C.A., Metcalf, M.R., Sellett, L.C., 1988. Evaluation of citrate-phosphate-dextrose-adenine as a storage medium for packed canine erythrocytes. Journal of Veterinary Internal Medicine 2, 126-132.

Sandler, S.G., Berry, E., Ziotnick, A., 1976. Benign hemoglobinuria following transfusion of accidentally frozen blood. Journal of American Medical Association 235, 2850-2851.

Sloan, T.B., Myers, G., Janik, D.J., Burger, E.M., Patel, V.V., Jameson, L.C. et al., 2009 Intraoperative autologous transfusion of hemolysed blood. Anesthesia and Analgesia 109, 38-42.

Sollberger, T., Walter, R., Brand, B., Contesse, J., Meredith, D.O., Reinhart, W.H., 2002. Influence of prestorage leucocyte depletion and storage time on rheologic properties of erythrocyte concentrates. Vox Sanguinis 82, 191-197.

Commentato [AL6]: Is this a web reference? Is it a book? How can readers access it?

448 Sowemimo-Coker, S.O., 2002. Red blood cell hemolysis during processing. Transfusion
449 Medicine Reviews 16, 46-60.
450
451 Spada, E., Perego, R., Baggiani, L., Proverbio, D., 2018. Haematological and morphological
452 evaluation of feline whole blood units collected for transfusion purposes. Journal of Feline
453 Medicine and Surgery 21, 732-740.
454
455 Spada, E., Proverbio, D., Baggiani, L., Bagnagatti De Giorgi, G., Ferro, E., Perego, R., 2017.
456 Change in haematological and selected biochemical parameters measured in feline blood
457 donors and feline whole blood donated units. Journal of Feline Medicine and Surgery 19,
458 375-381.
459
460 Spada, E., Proverbio, D., Bagnagatti De Giorgi, G., Perego, R., Valena, E., Della Pepa, A.,
461 Baggiani, L., 2015. Clinical and haematological responses of feline blood donors
462 anaesthetised with a tiletamine and zolazepam combination. Journal of Feline Medicine and
463 Surgery 17, 338-341.
464
465 Wardrop, K.J., Tucker, R.L., Mugnai, K., 1997. Evaluation of canine red blood cells stored in a
466 saline, adenine, and glucose solution for 35 days. Journal of Veterinary Internal Medicine
467 11, 5-8.
468
469 Weingart, C., Giger, U., Kohn, B., 2004. Whole blood transfusions in 91 cats: A clinical
470 evaluation. Journal of Feline Medicine and Surgery 6, 139-148.
471
472

Table 1 .

Effect of storage on hematological parameters in feline whole blood citrate phosphate dextrose adenine (CPDA) units collected with an open system for transfusion purposes.

Parameter (reference interval)	Day	*n* [a]	Mean (% change from D0)	SD	95% CI	Median (% change from D0)	Range Min	Range Max	95% CI	*P* [b]
Red blood cell count	0	12	6.6	1.0	5.9, 7.2	6.4	5.0	9.1	5.8, 7.2	-
(6.5-11.1 x10^{12}/L)	7	7	7.0 (+6.1)	0.7	6.3, 7.6	7.5 (+17.2)	5.7	7.6	6.1, 7.5	0.208
	14	7	6.9 (+5.0)	1.2	5.7, 8.1	6.8 (+6.3)	4.9	8.7	5.4, 8.4	0.199
	21	9	6.3 (-4.6)	0.8	5.7, 6.9	6.4 (0.0)	4.8	7.5	5.6, 7.2	0.108
	28	9	6.3 (-4.6)	0.7	5.7, 6.9	6.4 (0.0)	4.7	7.5	5.9, 6.9	0.194
	35	12	6.4 (-3.0)	1.0	5.8, 7.1	6.3 (-1.6)	4.8	8.8	5.8, 6.8	0.045
Hematocrit	0	12	25.1	4.8	22.0, 28.1	23.0	20.8	37.3	21.9, 28.5	-
(31.7-48%)	7	7	24.8 (-1.2)	2.2	22.7, 26.9	24.0 (+4.4)	22.7	28.7	22.7, 27.5	0.187
	14	7	25.5 (+1.6)	4.3	21.5, 29.5	24.6 (+7.0)	20.3	32.6	21.4, 31.4	0.156
	21	9	22.9 (-8.8)	2.9	20.6, 25.1	23.2 (+0.9)	19.7	28.5	19.9, 25.6	0.074
	28	9	23.1 (-8.0)	3.0	20.7, 25.4	23.1 (+0.4)	19.6	29.3	20.0, 24.8	0.460
	35	12	24.8 (-1.2)	5.3	21.4, 28.2	23.5 (+2.2)	19.4	35.6	20.4, 29.1	0.569
Hemoglobin	0	12	8.7	1.6	7.6, 9.7	8.1	7.0	12.6	7.4, 10.0	-
(10.6–15.6 g/dL)	7	7	8.7 (0.0)	0.8	7.9, 9.5	8.8 (+8.6)	7.7	10.1	7.9, 9.7	0.069

	14	7	8.7 (0.0)	1.7	7.1, 10.3	8.4 (+3.7)	6.7	11.2	6.8, 10.8	0.322
	21	9	7.9 (-9.2)	1.2	7.0, 8.9	7.6 (-6.2)	6.4	9.8	6.7, 9.3	0.645
	28	9	7.9 (-9.2)	1.2	7.0, 8.8	7.6 (-6.2)	6.4	9.8	6.6, 9.4	0.553
	35	12	8.3 (-4.6)	1.5	7.3, 9.3	8.1 (0.0)	6.4	11.2	6.8, 9.8	0.164
Mean cell volume	0	12	38.1	4.3	35.3, 40.9	39.3	30.6	44.4	34.1, 41.0	-
(36.7-53.7 fL)	7	7	35.5 (-6.8)	2.9	32.7, 38.2	34.6 (-12.0)	32.1	40.7	32.8, 39.5	0.889
	14	7	37.2 (-2.4)	5.3	32.2, 42.1	37.1 (-5.6)	30.7	44.0	30.7, 43.1	0.534
	21	9	36.4 (-4.5)	4.8	32.7, 40.0	35.1 (-10.7)	30.7	44.4	31.1, 41.3	0.245
	28	9	36.7 (-3.7)	5.1	32.8, 40.6	35.4 (-9.9)	30.5	45.4	31.1, 41.8	0.578
	35	12	38.5 (+1.1)	5.9	34.7, 42.3	38.2 (-2.8)	31.2	51.8	34.0, 40.7	0.649
Mean cell hemoglobin	0	12	13.2	2.0	12.0, 14.5	13.4	9.6	16.6	11.7, 15.1	-
(12.3-17.3 pg)	7	7	12.5 (-5.3)	1.5	11.1, 13.9	12.0 (-10.5)	10.8	15.5	11.2, 14.5	0.033
	14	7	12.7 (-3.8)	2.5	10.3, 15.0	12.8 (-4.5)	9.3	16.3	9.8, 15.7	0.366
	21	9	12.6 (-4.6)	1.9	11.2, 14.1	12.1 (-9.7)	10.1	15.8	11.0, 15.0	0.953
	28	9	12.6 (-4.6)	1.9	11.1, 14.1	11.9 (-11.2)	10.2	16.0	11.1, 14.9	0.836
	35	12	12.9 (-2.3)	1.8	11.7, 14.1	12.3 (-8.2)	10.3	16.5	11.5, 14.8	0.314
Mean cell hemoglobin concentration	0	12	34.7	1.8	33.5, 35.8	34.2	31.6	37.7	33.5, 36.8	-
(30.1-35.6 g/dL)	7	7	35.2 (+1.4)	1.4	33.9, 36.5	35.2 (+2.9)	33.7	38.0	33.8, 36.7	0.031

	14	7	34.0 (-2.0)	2.4	31.6, 36.3	34.0 (-0.6)	30.6	38.7	31.4, 36.6	0.578
	21	9	34.8 (+0.3)	2.7	32.7, 36.9	34.0 (-0.6)	31.8	40.6	32.9, 37.6	1.00
	28	9	34.3 (-1.2)	2.4	32.5, 36.2	33.5 (-2.1)	31.8	39.0	33.0, 37.5	0.425
	35	12	33.8 (-2.6)	2.1	32.4, 35.1	33.4 (-2.3)	31.5	38.0	32.1, 34.1	0.064
Red blood cell distribution width	0	12	17.8	2.2	16.4, 19.3	17.3	15.3	22.6	15.8, 19.7	-
(16.7-22.9%)	7	7	18.1 (+1.7)	1.6	16.5, 19.7	17.3 (0.0)	16.7	20.7	16.7, 20.5	0.812
	14	7	17.9 (+0.6)	1.4	16.6, 19.2	17.4 (+0.6)	16.7	20.8	16.8, 19.8	0.046
	21	9	18.5 (+3.9)	1.5	17.3, 19.7	18.5 (+6.9)	16.8	20.8	17.0, 20.5	0.020
	28	9	18.0 (+1.1)	1.8	16.6, 19.4	17.2 (-0.6)	15.9	20.4	16.0, 19.9	0.141
	35	12	18.8 (+5.6)	2.8	17.0, 20.6	18.1 (+4.6)	15.8	25.8	16.5, 20.6	0.024

SD, standard deviation; CI, confidence interval; Min, minimum percentage change from D0; Max, maximum percentage change from D0

[a] Number of samples analyzed at each sample time obtained at time of unit collection (D0), and every 7 days up to day 35 (D7, D14, D21, D28 and D35).

[b] *P*-value for paired differences (compared to D0)

Table 2.

Effect of storage on erythrocyte morphological index in feline whole blood citrate phosphate dextrose adenine (CPDA) units collected with an open system for transfusion purposes.

Day	*n* [a]	Mean (% change from D0)	SD	95% CI	Median (% change from D0)	Range		95% CI	*P* [b]
						Min	Max		
0	12	0.4	0.3	0.2, 0.7	0.3	0.1	1.2	0.2, 0.8	-
7	7	1.5 (+275)	0.07	1.4, 1.6	1.5 (+400)	1.5	1.6	-	0.062
14	7	1.3 (+225)	0.4	0.8, 1.7	1.3 (+333)	0.4	1.8	0.8, 1.8	0.046
21	9	1.5 (+275)	0.3	1.3, 1.8	1.5 (+400)	0.8	1.9	1.3, 1.8	0.003[c]
28	9	1.6 (+300)	0.3	1.4, 1.9	1.7 (+467)	1.1	2.0	1.3, 1.9	0.003 [c]
35	12	1.8 (+350)	0.2	1.6, 2.0	1.9 (+533)	1.1	2.1	1.8, 2.0	0.000 [c]

SD, standard deviation; CI, confidence interval; Min, minimum percentage change from D0; Max, maximum percentage change from D0

[a] Number of samples analyzed at each sample time obtained at time of unit collection (D0), and every 7 days up to day 35 (D7, D14, D21, D28 and D35).

[b] *P*-value for paired differences (compared to D0)

[c] $P < 0.01$

Table 3.

Effect of storage on percentage hemolysis in feline whole blood citrate phosphate dextrose adenine (CPDA) units collected with an open system for transfusion purposes.

Day	*n*[a]	Mean (% change from D0)	SD	95% CI	Median (% change from D0)	Range		95% CI	*P* [b]
						Min	Max		
0	12	0.3	0.1	0.2, 0.4	0.4	0.1	0.6	0.2, 0.5	-
7	7	0.6 (+100)	0.2	0.4, 0.8	0.7 (+75)	0.3	0.8	0.4, 0.8	0.010
14	7	0.6 (+100)	0.2	0.4, 0.8	0.7 (+75)	0.3	0.8	0.4, 0.8	0.012
21	9	0.9 (+200)	0.1	0.7, 1.0	0.9 (+125)	0.6	1.2	0.7, 1.0	0.000 [c]
28	9	1.1 (+267)	0.2	0.9, 1.3	1.0 (+150)	0.7	1.8	0.9, 1.3	0.000 [c]
35	12	1.2 (+300)	0.5	0.8, 1.5	1.1 (+175)	0.4	2.6	0.8, 1.5	0.001 [c]

SD, standard deviation; CI, confidence interval; Min, minimum percentage change from D0; Max, maximum percentage change from D0

[a] Number of samples analyzed at each sample time obtained at time of unit collection (D0), and every 7 days up to day 35 (D7, D14, D21, D28 and D35).

[b] *P*-value for paired differences (compared to D0)

[c] $P < 0.01$

Table 4.

Effect of storage on selected biochemical parameters in feline whole blood citrate phosphate dextrose adenine (CPDA)-1 units collected with an open system for transfusion purposes.

Parameter	Day	*n* [a]	Mean (% change from D0)	SD	95% CI	Median (% change from D0)	Range		95% CI	*P* [b]
							Min	Max		
Glucose	0	12	28.8	8.2	23.5, 34.0	28.8	14.8	48.0	26.2, 32.2	-
(4.4-6.1 mmol/L)	7	7	28.2 (-1.6)	7.8	20.9, 35.4	26.2 (-9.0)	22.4	45.5	23.0, 36.9	0.015
	14	7	23.5 (-18.4)	4.1	19.7, 27.3	21.7 (-24.5)	19.7	31.1	19.9, 28.4	0.015
	21	9	23.8 (-17.4)	6.7	18.7, 29.0	21.7 (-24.7)	17.8	39.5	19.2, 28.2	0.003 [c]
	28	9	22.8 (-20.8)	8.7	16.1, 29.5	23.5 (-18.4)	13.0	43.1	16.8, 25.2	0.003 [c]
	35	12	20.4 (-29.2)	8.4	15.0, 25.8	18.8 (-34.7)	12.4	43.3	13.7, 23.8	0.001 [c]
Sodium	0	11	181.9	7.4	176.9, 186.9	180.4	171.4	193.9	176.4, 188.7	-
(141-152 mmol/L)	7	7	180.9 (-0.6)	7.3	174.1, 187.6	181.8 (+0.8)	169.3	192.4	172.7, 188.5	0.349
	14	7	186.1 (+2.3)	9.4	177.4, 194.9	188.1 (+4.3)	173.9	197.2	175.6, 196.1	0.028
	21	9	189.4 (+4.1)	15.2	177.7, 201.1	185.1 (+2.6)	164.7	217.0	179.3, 202.9	0.053
	28	9	188.6 (+3.7)	7.4	182.8, 194.3	188.4 (+4.4)	176.7	202.8	182.5, 192.7	0.009 [c]
	35	11	186.1 (+2.3)	7.3	181.1, 191.0	182.8 (+1.3)	176.8	197.5	179.2, 192.1	0.029
Potassium	0	11	3.2	0.3	2.9, 3.4	3.2	2.6	3.8	3.0, 3.5	-
(3.7-5.8 mmol/L)	7	7	3.7 (+15.6)	0.3	3.4, 4.0	3.8 (+18.8)	3.3	4.2	3.3, 4.1	0.001 [c]

14	7	4.2 (+31.3)	0.2	3.9, 4.4	4.2 (+31.3)	3.9	4.8	3.9, 4.5	0.000 [c]
21	9	4.5 (+40.6)	0.5	4.1, 4.9	4.5 (+40.6)	4.1	5.7	4.1, 4.8	0.003 [c]
28	9	4.3 (+34.4)	0.4	4.0, 4.6	4.3 (+34.4)	3.6	5.0	4.2, 4.7	<0.0001 [c]
35	11	4.3 (+34.4)	0.3	4.1, 4.6	4.5 (+40.6)	3.7	4.9	4.0, 4.6	<0.0001 [c]

SD, standard deviation; CI, confidence interval; Min, minimum percentage change from D0; Max, maximum percentage change from D0

[a] Number of samples analyzed at each sample time obtained at time of unit collection (D0), and every 7 days up to day 35 (D7, D14, D21, D28 and D35).

[b] *P*-value for paired differences (compared to D0)

[c] $P < 0.01$

517

28

Figure legends

Fig. 1. Scatter diagram indicating the progressive increase in percentage hemolysis during storage for feline whole blood units collected with an open system for transfusion purposes. Dashed line represents the 1% hemolysis Food and Drug Administration (USA) limits for human transfusion medicine.

Fig. 2. Scatter diagram indicating the relationship between percentage hemolysis and morphological index for feline whole blood units collected with an open system for transfusion purposes. *n* = number of data pairs (number of samples).