Comp Clin Pathol (2014) 23:1395–1401
DOI 10.1007/s00580-013-1796-3

ORIGINAL ARTICLE

Laboratory analysis of canine packed red blood cells—effects of collection and processing on haemolysis, haemoglobin concentration, haematocrit and blood culture

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Received: 17 February 2013 / Accepted: 31 July 2013 / Published online: 18 August 2013

Abstract The aim of this study was to evaluate the relationship between blood collection and processing techniques and the quality of canine packed red blood cell (pRBC) units. This prospective study analysed 235 canine blood donations, followed by processing and evaluation of pRBC units. The need for sedation, number of venepunctures, whole blood volume, time to processing and time to analysis were registered, and five different centrifugation protocols were performed. The final pRBC volume, haematocrit, total haemoglobin concentration, haemolysis and bacterial contamination of pRBC units were evaluated. Obtained results were within the reference range of human blood banks' guidelines. One unit presented a positive bacteriologic analysis. No significant differences in haemolysis were detected when factors related to sedation, blood collection, time to processing or centrifugation protocols were studied. Significantly higher haematocrit values were detected in units centrifuged in a faster and longer programme (3,500×g, 15 min). There was a direct increase of haemolysis with longer time spent between centrifugation and analysis. This study demonstrates that haemolysis is significantly higher when analyses post centrifugations take 48–72 h to be performed than in short periods.

Keywords Blood collection · Processing · Packed red blood cells · Haemolysis · Quality control

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Introduction

Blood transfusions in dogs are an essential therapeutic complement of any veterinary intensive care unit. Previous studies in veterinary transfusion medicine were mainly centred in the transfusion recipient. However, proper care in this area must also rely in the donors' good health and the quality of blood products. Therefore, it is essential to use safe components, obtained according to studied protocols, in order to minimize the erythrocyte damage and to maintain the viability of blood components.

Packed red blood cells (pRBCs) are probably the most widely used component in canine haemotherapy. Although the best procedures for blood collection, processing and storage of canine pRBCs have been investigated by several authors (Gibson and Abrams-Ogg 2012; Feldman and Sink 2008; Ford and Mazzaferro 2006; Lucas et al. 2004; Schneider 1995), many critical points can still be identified and deserve to be addressed. The large variability in size,

Table 1 Reference values of the main parameters for assessing the quality of pRBCs, in additive solutions, in human blood banks that must be achieved in 75 % of the tested units

	European Union (Council of Europe 2011)	American Association of Blood Banks (Kakaiya et al. 2011)	UK Blood Transfusion Services (2005)
Volume (ml)	To be defined for the system used	N.D.	280±60
Haematocrit (%)	50–70	55–65	N.D.
Haemoglobin (g/unit)	>40	N.D.	>40
Haemolysis (%)[a]	<0.8 % of red cell mass	1 %	<0.8 % of red cell mass
Bacterial culture	Negative	Negative	Negative

N.D. not determined

[a] At the end of storage period

anatomical conformation and behaviour of canine blood donors implies that, in field conditions, it is often difficult to follow standardized collection procedures in all animals. An example of such difficulties is the frequent donor movement, with a consequent need for multiple venepunctures and the entry of air into the blood collection bag. In order to assure the quality of blood products collected under such conditions, it is very important to understand the influence of collection procedures in the final quality of pRBC units. Centrifugation is another critical point to ensure the quality of pRBCs. Different centrifugation velocities, previously described (Gibson and Abrams-Ogg 2012; Feldman and Sink 2008; Ford and Mazzaferro 2006; Lucas et al. 2004; Mathews et al. 2006; Schneider 1995), have an important potential influence in the extent of pRBC haemolysis, which is essential to evaluate in the search for the optimal protocol of this processing stage.

Human blood banks follow very strict quality control guidelines, in order to easily identify nonconformities during blood collection and processing. The final volume of pRBC units and their haematocrit (Hct), total haemoglobin concentration (Hb), haemolysis percentage and bacterial contamination are the most important parameters analysed in the daily routine. Human reference values published by regulatory institutions are displayed in Table 1 (Council of Europe 2011; Kakaiya et al. 2011; UK Blood Transfusion Services 2005).

To the authors' knowledge, no studies addressed the quality parameters of canine pRBCs in a commercial blood bank; therefore, specific guidelines for canine blood banks are inexistent. Previous experimental studies evaluated the effect of the additive solutions Adsol® (Fenwal Laboratories, Baxter Health Care Corporation, Deerfield, IL, USA) and Nutricel® (Miles Inc., Pharmaceutical Division, West Haven, CT, USA) in the preservation of pRBCs (Wardrop et al. 1994a, b, 1997). Their results are summarized in Table 2. The carefully controlled conditions of such experimental procedures, as well as the cautious selection of donors, looking for similar weights and uniform haematocrit values, and the evaluation of a small number of units hamper extrapolations to commercial blood banks (Wardrop et al. 1994a, b, 1997).

Although different protocols have been described for canine whole blood centrifugation in order to obtain pRBC and fresh frozen plasma units (Table 3), there are no studies comparing centrifugation protocols and the corresponding quality parameters of pRBCs. The increasing number of canine blood banks requires the establishment of standardized blood collection and processing methods in order to improve the quality and safety of pRBC units. This study intends to be the first step to help on the implementation of such guidelines. We evaluated the quality of pRBC units, based on their final volume, Hct, total Hb concentration, haemolysis and presence of aerobic and anaerobic bacterial contamination, and searched for its relationship with the blood collection and processing procedures.

Table 2 Effect of additive solutions in the quality parameters of canine pRBCs

	Wardrop et al. (1994a)	Wardrop et al. (1994b)	Wardrop et al. (1997)
Additive solution	Adsol®, Nutricel®	Adsol®	Nutricel®
No. of pRBC units	6/6	6	6
Volume (ml)	N.D.	N.D.	N.D.
Haematocrit (%)	60±2/65±4	65±9	63±2
Haemoglobin (g/dl)	23.2±2.0/23.3±1.8	N.D.	N.D.
Haemolysis (%)	0.03±0.01/0.06±0.03	0.18±0.09	0.07±0.04
Bacterial culture	N.D.	N.D.	N.D.

N.D. not determined

Table 3 Published protocols for canine whole blood centrifugation

	Speed	Duration (min)	Deceleration	Temperature (°C)
Kerl and Hohenhaus (1989)	5,000×*g*	25	–	1–6
Wardrop et al. (1994a, b, 1997)	4,050×*g*	6	–	4
Schneider (1995)	5,000×*g*	5	Slow	–
Chiaramonte (2004)	4,100 rpm	10	–	4
Roberts et al. (2004)	5,000×*g*	5	–	6
Lucas et al. (2004)	4,000 rpm	15	3.5 min	10
Ford and Mazzaferro (2006)	4,000–5,000×*g*	5	–	–
Mathews et al. (2006)	2,000×*g*	10	–	–
Feldman and Sink (2008)	5,000×*g*	5	–	1–6

Materials and methods

The study protocol was approved by the ICBAS-UP Ethics Committee (project number 008/2012). Two hundred and thirty-five whole blood units were collected from 54 randomly selected healthy mixed-breed dogs, weighing up to 60 kg. In order to evaluate the technical difficulties associated with the processing of low-volume whole blood units, which could affect the quality of pRBCs, donors with more than 15 kg were included. All animals had been vaccinated and dewormed and had negative PCR annual analysis (Idexx Laboratories, Barcelona, Spain) for the following agents: *Anaplasma* spp., *Ehrlichia* spp., *Babesia canis*, *Leishmania infantum* and *Dirofilaria immitis*. CBC and chemistry profiles were within normal reference ranges; pre-donation Hct was 38–57 % (mean 47.6, SD±4.5). Blood collections were held under the regular donation programme from a blood bank and performed in several veterinary clinics and hospitals, and placeholders were provided by owners. Data were collected for 2 years, and blood donation frequency was variable, ranging from one to seven blood collections per year, with a minimum period of 2 months between donations.

Commercial triple pack collection systems were used (Laboratorios Grifols SA, Barcelona, Spain), consisting of a primary bag for the collection of 450 ml±10 % of blood with 63 ml of citrate phosphate dextrose (CPD) anticoagulant (trisodium citrate, sodium phosphate and dextrose), a secondary bag with 100 ml additive solution Adsol (sodium chloride, dextrose, adenine and mannitol) and an empty bag for plasma storage. No leukocyte depletion filters were used. When the estimated collected volume was less than 405 ml, a proportional volume of CPD was aseptically removed, using a sterile connection (CompoDock, Fresenius SE, Germany) with a transfer bag, in order to ensure the same proportion between CPDA and whole blood volumes. Blood collection was performed according to the following protocol: a complete physical exam was performed followed, if necessary, by sedation with the intravenous administration of 0.5 mg/kg diazepam (Roche Products Inc., Basel, Switzerland) and 5 mg/kg ketamine (Vétoquinol SA, Lure, France). Donors were placed in lateral recumbency, followed by local trichotomy and aseptic preparation (using chlorhexidine and alcohol) of the puncture site. Jugular venepuncture allowed blood to flow by gravity into the collection bag, which was gently inverted to mix the blood with the anticoagulant at every 50 ml of collected blood. If a halt of blood flow was noted, the needle was immediately and carefully removed from the puncture site, avoiding any contact with non-sterile surfaces. The blood in the collection system tube was allowed to flow into the primary bag, in order to contact with the anticoagulant, resulting in an open collection system. In these cases, a second venepuncture was performed on the contralateral jugular vein with the same needle and a similar aseptic preparation. The collected blood volumes were between 13 and 15 % of the donors' total blood volumes, calculated on the basis of the dogs' weight, assuming that canine total blood volume is 85 ml/kg (Jahr et al. 2008) and that 1 ml of whole blood weighs 1.053 g (Kakaiya et al. 2011). Once the total volume was obtained, the collection tube was sealed (Composeal, Fresenius SE, Germany). The duration of the collection process was 5–15 min. Whole blood units were immediately stored at 6±2 °C and processed within 24 h. Units collected until 12 a.m. were processed during the afternoon (belonging to <12-h group), while those collected from 12 a.m. until 7 p.m. were processed in the afternoon the next day (12–24-h group).

A gentle stir of each unit was performed before processing, followed by proper packaging of triple bag systems into the centrifuge cups (Megafuge 40R, Thermo Scientific, Waltham, MA, USA), avoiding plastic folds or empty spaces and ensuring a symmetrical distribution of the weight with a tolerated difference of 0.2 g between opposite cups. These procedures avoid oscillations during centrifugation, thus allowing for a better cellular sedimentation. Units were centrifuged at 6 °C. Five different centrifugation protocols were randomly performed: 2,000×*g* for 10 min (42 units), 15 min (37 units) and 20 min (57 units), with 80 s of acceleration and 110 s of deceleration and 3,500×*g* for 10 min (73 units) and 15 min (26 units), with similar times of acceleration and deceleration.

Since sudden changes in centrifugation speed are associated with higher haemolysis (Sowemimo 2002), rapid accelerations and decelerations were avoided. Protocols were selected based on previous canine reports. Supernatant plasma was then separated into the transfer bag, using a manual plasma extractor. The desired volume of Adsol was added to the primary pRBC bag, based on a proportion of 100 ml Adsol to 450 ml of whole blood. For quality analysis, an 8-ml pRBC aliquot was aseptically separated, using a sterile connection (CompoDock, Fresenius SE, Germany), from the pRBC bag to a sample bag (Macopharma, Mouvaux, France).

Aliquots from pRBC units were analysed within 72 h. Analysis were performed twice a week (Tuesday and Friday), while blood processing could be made every day. In order to evaluate the influence of time spent from processing to analysis, three different groups were established (<24, 24–48 and 48–72 h). Haematocrit was obtained using a microhaematocrit centrifuge according to standard methodology (Brown 1984). The percentage of haemolysis was obtained using the following formula (Sowemimo 2002):

$$\%\ Haemolysis = Plasma\,supernatant\,haemoglobin(\mathrm{g/L}) \times (100-Hct)/Total\,haemoglobin(\mathrm{g/L})$$

Plasma supernatant haemoglobin was determined by spectrophotometry using HemoCue Plasma Low Hb (HemoCue Inc., Lake Forest, CA, USA) after centrifugation (centrifuge IEC Centra CL3R, Thermo Scientific, Waltham, MA, USA) according to the manufacturer's protocol. Total Hb was measured using a COULTER LH 750 analyser (Beckman Coulter, Brea, CA, USA) according to the manufacturer's protocol. Aerobic and anaerobic bacterial contamination was investigated by adding a 5-ml pRBC sample to culture bottles, under sterile conditions, containing a specific growth medium (BacT/ALERT PF, bioMérieux, Marcy l'Etoile, France), followed by incubation at 37 °C and continuous examination for 7 days using the analyser BacT/ALERT 3D (bioMérieux, Marcy l'Etoile, France).

Results were analysed using SPSS statistics 17.0 program (IBM Corporation, Chicago, IL, USA) (Ott and Longnecker 2010). Normal distribution of data was assessed by the Kolmogorov–Smirnov test. One-way analysis of variance (ANOVA) was used to compare haemolysis values of pRBCs according to the original whole blood unit volume, pRBC haematocrit, time from processing to quality control analysis and different centrifugation protocols. ANOVA was also used to compare haematocrit values measured in pRBCs centrifuged according to five different protocols. Specific significant differences between groups were identified by a post hoc Tukey's test. Student's two independent samples t test was used to compare haemolysis values according to the use of a sedative protocol, number of venepunctures and the time from blood collection to processing. The critical probability for statistical significance was assumed to be $p \leq 0.05$.

Results

A total of 235 whole blood units were collected with a mean volume of 349 ml, ranging from 218 to 580 ml. Twenty-two collections (9.4 %) required two venepunctures, allowing air to enter into the collection bag, resulting in an open collection system. Ninety-six low-volume whole blood units, with less than 300 ml, were collected from smaller donors weighing 15–25 kg. After processing, the mean volume of the pRBC units was 251.8 ml (SD±70.9), with a mean Hct of 59.3 % (SD±4.3) and a mean total haemoglobin of 21.2 g/dl (SD± 1.5). The mean haemolysis was 0.23 % (SD±0.17). Only one sample (0.36 %) presented a positive bacteriologic culture due to contamination with *Enterobacter cloacae*. As this unit resulted from a multiple-venepuncture collection, it represents 4.5 % of the 22 units collected by multiple venepunctures.

Units collected from sedated ($n=157$) and non-sedated donors ($n=78$) revealed similar haemolysis values (Fig. 1). No significant differences were registered between haemolysis of pRBCs following one or two venepuncture collections, despite a slightly higher haemolysis in the latter group (Fig. 2), neither between different whole blood unit volumes (Fig. 3). Packed red blood cell units processed until 12 h post collection revealed a slightly lower mean haemolysis (0.22 %, SD 0.16) than those that rested for 12–24 h (0.25 %, SD 0.22), although of no statistical significance (Fig. 4). A progressively higher haemolysis was found with higher velocities and centrifuge time, increasing from 0.18 % (SD 0.15) with 2,000×g centrifugation for 10 min, to 0.24 % (SD 0.20) with 2,000×g centrifugation for 20 min, and to 0.27 % (SD 0.21) with 3,500×g centrifugation for 15 min (Fig. 5). However, no statistically significant differences were found. A total of 10 units centrifuged at 2,000×g for 10 min (6 units) and

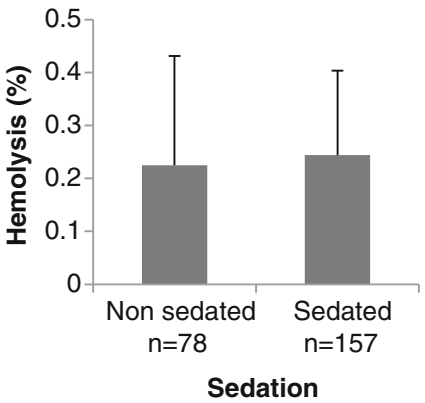

Fig. 1 Mean haemolysis values (±SD) of pRBCs according to the use of sedative protocol during blood collection. $p>0.05$

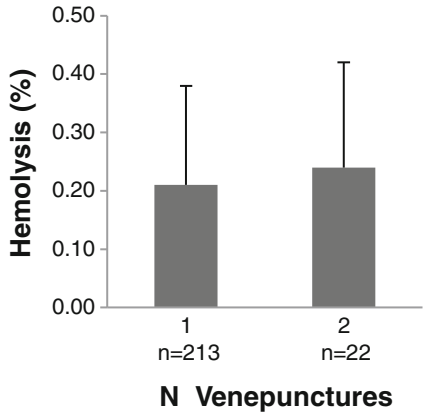

Fig. 2 Mean haemolysis values (±SD) of pRBCs according to the number of venepunctures needed for whole blood collection. $p > 0.05$

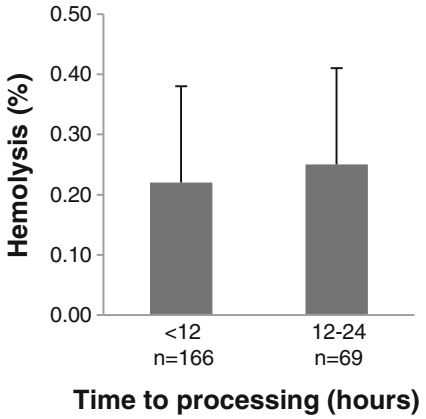

Fig. 4 Mean haemolysis values (±SD) of pRBCs according to time from blood collection to processing. $p > 0.05$

15 min (4 units) had to be excluded from this study, due to incomplete plasma separation, and were not accounted on any reported value. Haematocrit values from pRBC units were also compared between centrifugation protocols (Fig. 6), revealing significant differences between centrifugations at 2,000×*g* for 10 min and 3,500×*g* for 15 min (58.13 % (SD 4.76) and 61.29 % (SD 4.22), respectively). Mean haemolysis values of pRBCs increased slightly with progressively higher haematocrits, from 0.21 % (SD 0.17) in units with Hct <56 % to 0.26 % (SD 0.14) in those with Hct ≥65 % (Fig. 7); differences were not statistically significant. Time from processing to analysis also significantly influenced haemolysis, with units analysed 48–72 h after the processing having higher haemolysis (0.28 %, SD 0.17) than those analysed in the first 24 h (0.18 %, SD 0.15) (Fig. 8).

Discussion

In this study, donors with a weight of more than 15 kg were accepted, because there is no clinical reason to exclude dogs weighing less than 25 kg, as widely advocated in most blood banks. If a blood bank has a technical capacity to aseptically prepare collection systems with lower anticoagulant volume, lower blood volumes can also be collected from smaller donors donating 13–15 % of TBV.

This study was performed using whole blood units collected under field conditions. The avoidance of sedation, whenever possible, led to the need for two venepunctures in some collections, following needle misplacement due to donors' movements. However, technicians were able to properly restrain canine donors in the majority of collections, justifying the infrequent need for multiple venepunctures ($n = 22$). This fact created the opportunity to study the consequences of this contingency in the characteristics of the corresponding pRBCs, not approached by previous studies. The single case of contamination in multiple-venepuncture collections suggests that the prompt rejection of units collected in open systems could result in a waste of blood products, considering that most of them seem to be sterile and, therefore, retain therapeutic value. Despite the high sensitivity of BacT/ALERT 3D analyser (bioMérieux, Marcy l'Etoile, France), found to be less than 1 colony forming unit/ml (Brecher et al. 2005), further studies are

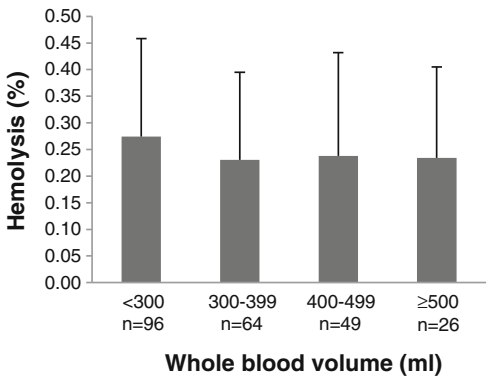

Fig. 3 Mean haemolysis values (±SD) of pRBCs according to the original whole blood unit volume. $p > 0.05$

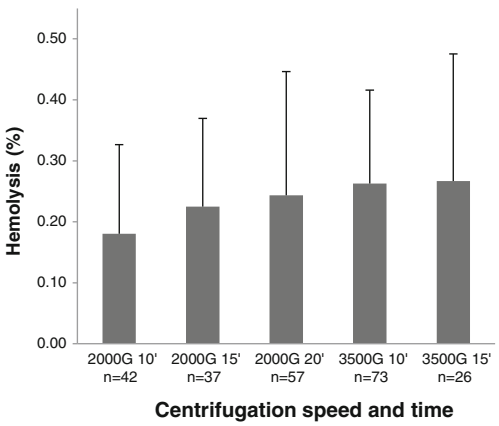

Fig. 5 Mean haemolysis values (±SD) measured in pRBCs centrifuged according to five different protocols. $p > 0.05$

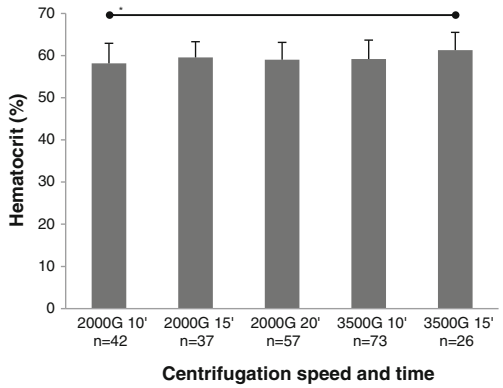

Fig. 6 Mean haematocrit (±SD) measured in pRBCs centrifuged according to five different protocols. *$p \leq 0.05$

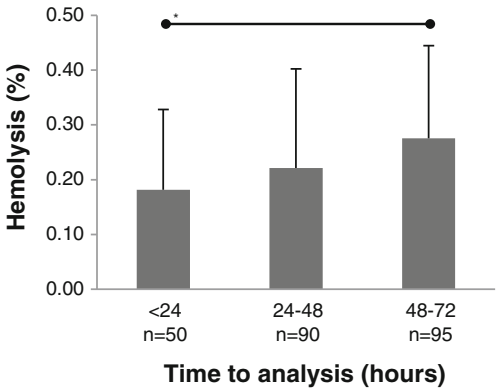

Fig. 8 Mean haemolysis values (±SD) of pRBCs according to time from processing to quality control analysis. *$p \leq 0.01$

needed to confirm that it is safe to administer pRBCs collected in open systems, due to false-negative blood cultures at the beginning of storage period (Schmidt et al. 2011).

To the authors' knowledge, there are no studies regarding the effects of sedation, used in canine donors, on pRBC haemolysis. Our study did not reveal any change in pRBC haemolysis, coming from sedated and non-sedated dogs, which may be due to low hemodynamic induced effects. Further studies are needed to clarify this issue. Mean haemolysis was higher in units that required multiple venepunctures, perhaps due to increased collection times or erythrocyte flow turbulence inside the collecting system, potentially resulting in the formation of blood clots.

The authors did not find any published study in canine practice supporting the obligation of a maximum period of 12 h from blood collection till processing. Therefore, we followed the human guidelines that advise the processing of whole blood units until 24 h post blood collection (Council of Europe 2011; Kakaiya et al. 2011; UK Blood Transfusion Services 2005). There was no statistically significant haemolysis differences between units processed until 12 h (0.22 %, SD 0.16) and those processed after 12–24 h (0.25 %, SD 0.16) (Fig. 4); however, as the standard deviation is large, further studies are needed to clarify this relationship.

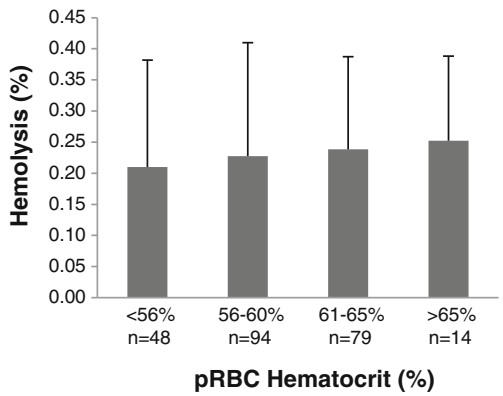

Fig. 7 Mean haemolysis values (±SD) of pRBCs according to their haematocrit. $p > 0.05$

Haemolysis appears to increase with speed and time of centrifugation. Although this observation seems to indicate that lower centrifuge velocities and durations would lead to better-quality pRBC units, incomplete plasma separation was found in six and four whole blood units, after centrifugations of 2,000×*g* for 10 min and 2,000×*g* for 15 min, respectively, which makes these protocols not advisable to obtain pRBCs. Considering that both 3,500×*g* protocols resulted in higher mean haemolysis than the 2,000×*g* protocols and that the protocol of 2,000×*g* for 20 min always resulted in complete plasma separation, the latter seems to be the best option. Proper packaging was harder to accomplish in low-volume whole blood units, due to high number of plastic folds, which may explain the higher, although not significant, mean haemolysis in the lower-volume pRBC units. Higher haematocrit units' tendency to present higher mean haemolysis percentages may be explained by faster centrifuge spin and lower viability of hard-packed RBCs (Ott and Longneckers 2010; Sowemimo 2002).

In this study, slower centrifuge rates than those described in majority of veterinary reports were used, in order to avoid exposing erythrocytes to stronger forces that could promote their lysis. It is not surprising, therefore, that the pRBC haematocrit values (mean 59.3 %, SD 4.6) and Hb concentrations (mean 21.2 g/dl, SD 1.5) were slightly lower than those previously published (Wardrop et al. 1994a, b, 1997). However, Hct results are within the human Council of Europe (2011) reference values (50–70 %), suggesting that it is a good option not to use higher centrifuge velocities.

Although human transfusion guidelines require a minimum Hb concentration of 40 g/unit in 80 % of the pRBC units (Council of Europe 2011; UK Blood Transfusion Services 2005), the variable volume of pRBC units in veterinary medicine precludes the definition of a minimum Hb concentration per unit. Considering that the minimum acceptable volume of human pRBC units is 220 ml (Council of Europe 2011; UK Blood Transfusion Services 2005), it is possible to calculate a minimum acceptable Hb concentration of 18.2 g/dl. In this study, 229 (97.5 %) pRBC units contained a higher

concentration than this value, once again validating the proposed centrifugation protocols.

The higher haemolysis associated to increased times until laboratory analysis may be associated with storage erythrocyte lesions, due to glucose consumption and consequent decrease of the ATP levels (Wolfe 1985).

The mean haemolysis percentage (0.23 %, SD 0.17) described in this study is higher than that described in previous canine pRBC reports (Wardrop et al. 1994a, b, 1997). Differences may be related to the number of analysed units. Large samples collected in field conditions, as in this study, imply more donors' variability. Moreover, the non-hospital conditions in which some collections were performed brought additional difficulties that may have increased the units' haemolysis.

Acceptable levels of free haemoglobin, induced by haemolysis of the transfused pRBCs, have not been established in dogs. Free haemoglobin in the body dissociates into α dimmers, which have to bind to haptoglobin to be removed by the reticuloendothelial system (Sowemimo 2002). In an adult human, 3 g of free haemoglobin (10 units of pRBCs with 0.5 % haemolysis each) can be transfused without the occurrence of haemoglobinuria (Sowemimo 2002). However, the occurrence of life-threatening acute transfusion reactions in dogs, with haemolysis likely related to inappropriate storage, was recently reported (Patterson et al. 2011), although the haemolysis values of the administered units were not reported. Further studies are needed to understand the degree of pRBC haemolysis that can be safely administrated to dogs.

This study demonstrates that pRBC haemolysis is significantly influenced by the time spent from processing to analysis. Although units with lower volume, higher haematocrit, longer time to processing and longer time to analysis tended to present higher haemolysis, there was no significant difference observed. Furthermore, the centrifugation protocol did not significantly influence haemolysis percentage, although it tended to increase with higher centrifugation speed and time. This study suggests that pRBC units collected in open systems may be safe to be transfused, although further studies are needed to confirm this statement, searching for false-negative blood cultures by repeating them at the end of the storage period.

Acknowledgments The authors are grateful to the Instituto Português do Sangue e da Transplantação, Hospital Veterinário do Porto and the Portuguese Foundation for Science and Technology (grant SFRH/BD/43946/2008) for their support to this project.

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