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Randomized double-blinded clinical trial on acute transfusion reactions in dogs receiving leukoreduced versus nonleukoreduced packed red blood cells

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Abstract

Background: Leukoreduction of blood products is commonly performed in human medicine, but its effect on outcome or incidence of transfusion reactions (TRs) in dogs is unknown.

Objectives: To prospectively evaluate the incidence of acute TRs in, and the outcome of, dogs receiving either leukoreduced (LR) or nonleukoreduced (N-LR) packed red blood cells (PRBC).

Animals: Dogs (n = 194) administered PRBC between August 2017 and June 2020.

Methods: Prospective randomized double-blinded clinical trial. Dogs were randomized to receive either LR or N-LR PRBC and clinicians, nurses and investigators were blinded to the group allocations. The incidence of TRs, change in PCV, hospitalization duration, and survival to discharge were recorded.

Results: Out of the 194 dogs, 96 received LR and 98 received N-LR PRBCs. The mean 12-hour change in PCV value was +9.22% (SD 5.27%) for dogs that received N-LR and +10.69% (SD 6.44%) for dogs that received LR PRBC (effect size 0.26, 95% confidence interval [CI] −0.02 to 0.55), which was not significantly different ($P = .08$). TRs were documented in 16/194 (8.24%) dogs, with 1/194 (0.51%) being a mild allergic reaction, while 15/194 (7.73%) had suspected febrile nonhemolytic TRs (FNHTRs). FNHTR incidence was not significantly different between the LR (6/96, 6.25%, 95% CI 2.8-13.56) and N-LR (9/98, 9.18%, 95% CI 4.92-17.11) groups ($P = .81$). Of the 156 dogs that survived to discharge, 80/156 received N-LR PRBC and 76/156 received LR PRBC which was not significantly different ($P = .66$).

Conclusions and Clinical Importance: A clinical advantage of using LR over N-LR PRBC in terms of TRs and increase in PCV after transfusion was not detected.

Abbreviations: DEA, dog erythrocyte antigen; FNHTR, febrile nonhemolytic transfusion reactions; HLA, human leukocyte antigen; HPA, human platelet antigen; ICU, intensive care unit; IMHA, immune mediated hemolytic anemia; IMTP, immune mediated thrombocytopenia; IQR, interquartile range; LR, leukoreduced; N-LR, nonleukoreduced; OR, odds ratio; PRBC, packed red blood cells; TACO, transfusion-associated circulatory overload; TR, transfusion reactions; TRALI, transfusion related acute lung injury; ULR, universal leukoreduction; VGEF, vascular endothelial growth factor.

KEYWORDS
anemia, febrile non-hemolytic transfusion reactions

1 | INTRODUCTION

Blood product transfusions are not benign interventions. In people who receive blood product transfusions, the incidence of acute transfusion reaction (an adverse reaction occurring within the first 24 hours of transfusion administration) is between 0.04% and 1.8%,[1–5] with febrile nonhemolytic transfusion reactions (FNHTRs) being the most common transfusion reaction (TR) observed.[3,4,6–8] Leukocytes and leukocyte-derived cytokines in blood products are suggested to be involved in the pathogenesis of FNHTRs.[9–14] Leukoreduction of blood products involves the removal of leucocytes from blood products using either a specific leukoreduction filter or apheresis and can be performed either before or after storage.[15] Prestorage leukoreduction of blood products reduces the presence of inflammatory cytokines in blood products, as well as the formation and proinflammatory properties of microvesicles during storage of packed red blood cells (PRBC).[16] In human medicine leukoreduction decreases immunization against human leukocyte antigen (HLA) and human platelet antigen (HPA), decrease platelet refractoriness and decrease the risk of FNHTR, prevent cytomegalovirus infections and improves clinical outcome in patients undergoing cardiac surgery.[6,17] Leukoreduction partially alleviates many of the storage related modifications ("storage lesion") of red blood cells in PRBC products.[18]

The benefits of leukoreduction in preventing transfusion-related acute lung injury (TRALI), death, noninfectious complications or transfusion immunomodulation are inconsistent.[6,17,19]

The cost-effectiveness of leukoreduction in human medicine is also debated.[17,20,21]

In dogs, the reported incidence of TRs varies between 4.18% and 25.63%, with no clear differentiation between acute and delayed TRs.[22–25] Prestorage filter leukoreduction of canine blood effectively removes leucocytes from whole blood and PRBC[26] and reduces cytokine accumulation, erythrocyte hemolysis and the release of vasoactive and procoagulant compounds.[26–29] However, leukoreduction of canine blood products is not commonly performed in veterinary medicine currently[30] and its effect on transfusion recipient outcome or incidence of TRs in dogs is unknown.

The aim of this study was to evaluate the incidence of acute TRs in, and the outcome of, dogs receiving either leukoreduced (LR) or nonleukoreduced (N-LR) PRBC transfusions. Our null hypothesis was that there would be no difference in the incidence of TRs or outcome between the 2 groups.

2 | MATERIALS AND METHODS

This was a double-blinded randomized clinical trial. Dogs requiring a blood transfusion over a 35-month period were prospectively enrolled in the study. Dogs that had had a previous blood product transfusion, required more than 1 unit of PRBC or another blood product within 24 hours or were lost to follow up in the first 24 hours after transfusion were excluded. The randomization was performed using a password protected document in Excel (Microsoft Office Excel 2016, Microsoft Corp.). The authors, attending clinicians and nursing staff were blinded to the treatment groups. The leukoreduction was performed before centrifugation of whole blood and further processing and storage using a quad bag system with an integrated leukoreduction filter (Composelect Blood Bag, Fresenius Kabi, Bad Homberg, Germany). The PRBC units were stored for a maximum of 35 days.

Cause of anemia, PCV before transfusion, transfusion volume, PRBC unit age, the documentation of any TR (including details of any treatment), PCV 12 hours after the end of the transfusion and survival to discharge were recorded.

The recipients and donors were blood typed using a commercial immunochromatographic test (Lab test QuickTest dog erythrocyte antigen [DEA] 1, Alvedia, Limonest, France).

A standard transfusion monitoring sheet was used during the transfusion, to record temperature, pulse rate, respiration rate, mucous membrane color, capillary refill time, urine, and serum color (if applicable), and the appearance and demeanor of the animal at least hourly, with a baseline assessment performed before starting the transfusion (Data S1). The TRs observed were recorded on the transfusion monitoring sheet if they occurred during the transfusion, or in the hospital record sheet, when observed after the end of the transfusion. The transfusions were administered using an infusion pump (Alaris GW, version V3) at 0.5-1 mL/kg/hour for the first 30 minutes and then the rate increased to a rate determined by the attending clinician. After the end of the transfusion the dog was monitored as required by the attending clinician with a minimum of once daily full physical examination including body temperature, pulse, and respiration assessment for 24 hours to assess for the presence of any acute TR. Recipient PCV was measured 12 hours after the end of the transfusion when possible.

If an acute TR was suspected during blood administration, the transfusion was stopped at the clinician's discretion while investigating the cause of TR. Acute development of urticaria, angioedema or pruritus during the transfusion was recorded as a suspected allergic reaction. If a recipient had an increase in rectal temperature of greater than 1°C from baseline, nonpathological reasons including external warming and recovery from general anesthesia were considered. If no such reason was found, then recipient serum was checked for hemolysis (as was recipient urine, if available) and, if present, a hemolytic transfusion reaction was suspected. If absent, the blood product was cytologically examined for bacteria and cultured. If abnormalities were noted, then a suspected septic transfusion was recorded. If neither a septic nor an acute hemolytic transfusion reaction was suspected,

then a FNHTR was recorded. If a dog developed respiratory distress (defined as increased effort and respiratory rate >40 breaths per minute) during or within 24 hours after the transfusion, transfusion-associated circulatory overload (TACO) was recorded if echocardiography performed by the attending clinician demonstrated an enlarged left atrium (with a left atrium-to-aorta ratio >1.5) and the respiratory distress resolved with treatment with administration of furosemide. Other causes of respiratory distress, including transfusion-related acute lung injury (TRALI) were investigated if TACO was not diagnosed. Any other abnormalities that occurred during the transfusions including vomiting were recorded. Total hospitalization duration, ICU duration of stay and survival to discharge were all recorded. Acute patient physiologic and laboratory evaluation ($APPLE_{FAST}$) scores were calculated using previously a published model.[31]

A sample size calculation was performed using the previously reported incidence of FNHTR in dogs of 24.17%.[25] In order to detect a 65% reduction in the incidence of FNHTRs[32,33] with a 1-sided significance of 0.05 and a power of 0.8, a minimum of 172 dogs in total (86 within each group) were required.

Statistical analyses were performed using the statistical software IBIM SPSS Version 26. The distribution of data was assessed for normality using the Shapiro-Wilk test. Data were analyzed and presented as mean ± SD for normally distributed data and median ± interquartile range (IQR) for nonnormally distributed data. A chi-square test was performed to determine the difference in the rate of TR between the LR and N-LR groups. A 1-sample *t*-test was used for normally distributed data and the Mann-Whitney *U* test was used for data that was not normally distributed. Statistical significance was set at $P < .05$. The Cohen's *d* effect size and its associated 95% confidence interval (CI) was calculated for normally distributed data.

Ethical approval was granted for this study by the university teaching hospital Ethics and Welfare committee (reference number URN M2015 0054).

3 | RESULTS

One hundred ninety-four dogs were enrolled on the study between August 2017 and June 2020, out of which 96/194 received (49.48%) LR PRBC and 98/194 (50.51%) N-LR PRBC. Although 200 dogs were intended to be recruited, 6 dogs were excluded from the study after its conclusion due to missing data on whether the PRBC units were LR or N-LR.

The most common breeds were crossbreeds (26/194, 13.40%), Labrador retrievers (18/194, 9.27%), cocker spaniels (16/194, 8.24%), English springer spaniels (10/194, 5.15%), and bichon frises (10/194, 5.15%). Out of the 194 dogs, 76 (39.17%) were female neutered, 73 (37.62%) were male neutered, 27 (13.91%) were intact males and 18 (9.27%) were intact females. The mean age at the time of transfusion therapy was 7.71 years (SD 3.23 years) and the median body weight was 16.70 kg (IQR 18.33).

The most common causes of anemia were primary immune mediated hemolytic anemia (IMHA) (69/194, 35.56%), neoplasia (41/194, 21.13%), primary immune mediated thrombocytopenia (IMTP) (21/194, 10.82%), gastrointestinal hemorrhage (15/194, 7.73%), and trauma (7/194, 3.60%).

There was no significant difference between the LR and N-LR groups in the prevalence of immune mediated disease (42/96, 43.75%; 48/98, 48.97%; $P = .46$). The presence of immune-mediated disease was not associated with an increased likelihood of acute TR (odds ratio [OR] 0.81, 95% CI 0.46-1.42; $P = .86$).

Dog erythrocyte antigen 1 type matched blood was administered in 179/194 (92.26%) cases, and 6/194 cases (3.09%) received a blood product of a different blood type. This information was not recorded in 9/194 (4.63%) of the cases. There was no significant difference in the number of non-DEA 1 type matched blood transfusions between the LR vs N-LR groups (3/96, 3.12%; 3/98, 3.06%; $P = .95$).

The volume of PRBC transfused was recorded for 178/194 cases and the median value was 14.88 mL/kg (IQR 8.79 mL/kg) for all cases, 14.04 mL/kg (IQR 8.14) for dogs that received N-LR PRBC and 16.67 mL/kg (IQR 9.92) for dogs that received LR PRBC. There was no significant difference in the volume of transfused RBC between dogs that received LR vs N-LR PRBC ($P = .14$). The mean rate of the PRBC infusion was 4.96 hours (SD 1.69 hours) and there was no significant difference in the rate of infusion between the LR vs N-LR groups ($P = .62$) (Table 1).

The age of the PRBC units at the time of transfusion was recorded in 186/194 (95.87%) dogs and the mean value was 15.57 days (SD 8.04). Dogs that had a documented FNHTR received PRBC units with a mean age of 16.33 days (SD 6.16), while dogs with no documented FNHTR received PRBCs with a mean age of 15.49 days (SD 8.22) which was not significantly different ($P = .67$). There was also no significant difference in the age of PRBC units received between dogs that survived to discharge and dogs that did not ($P = .74$). There was no significant difference in the age of PRBC units administered to the LR and N-LR groups ($P = .44$) (Table 1). The effect size was −0.11 (95% CI −0.39 to 0.17).

The PCV value before transfusion was collected for 192/194 cases and the median value was 15% (IQR 6%). There was no statistically significant difference in the PCV value measured before transfusion between dogs that received LR vs N-LR PRBC ($P = .88$). The PCV value 12 hours after transfusion was collected for 177/194 cases and the median value was 25% (IQR 9%) for all cases, 25% (IQR 9%) for dogs that received N-LR PRBC and 25% (IQR 9%) for dogs that received LR PRBC. The PCV value was collected at a median of 12 hours after transfusion (IQR 1 hour). There was no statistically significant difference in the PCV after transfusion between dogs that received LR vs N-LR PRBC ($P = .56$). The increase in PCV value approximately 12 hours after transfusion was recorded in 178/194 cases and the mean value was +9.68% (SD 5.77%) for all cases, +9.22% (SD 5.27%) for dogs that received N-LR PRBC and +10.69% (SD 6.44%) for dogs that received LR PRBC. There was no statistically significant difference in the increase in PCV value between dogs that received LR vs N-LR PRBC ($P = .08$) (Table 1). The effect size was 0.26 (95% CI −0.02 to 0.55).

Fifteen dogs (15/194, 7.73%) had a suspected FNHTR, 14/15 (93.33%) during the transfusion and 1/15 (6.66%) 12 hours after

Variable	LR group Median (IQR)	N-LR group Median (IQR)	*P* value
Volume of PRBC	16.67 mL/kg (9.92)	14.04 mL/kg (8.14)	.14
Rate of PRBC infusion	5.08 hours (1.71)	4.81 hours (1.66)	.62
PCV value before transfusion	15% (5.05%)	15% (6.01%)	.88
PCV value after transfusion	25% (9%)	25% (9%)	.56
Days of hospitalization	6 days (4)	5 days (4)	.06
Days of ICU hospitalization	2 days (2)	1 day (2.11)	.39
Variable	**LR group Mean (SD)**	**N-LR group Mean (SD)**	***P* value**
Age of PRBC	15.11 days (7.94)	16.02 days (8.14)	.44
Increase in PCV 12 h after transfusion	10.69% (6.44%)	9.22% (5.27%)	.08
$APPLE_{FAST}$ score	25.27 (5.73)	24.28 (5.21)	.26
Variable	**LR group Number, % (95% CI)**	**N-LR group Number, % (95% CI)**	***P* value**
Incidence of FNHTRs	6/96, 6.25% (2.8-13.56)	9/98, 9.18% (4.92-17.11)	.81
Survival to discharge	76/96, 79.17% (69.68-86.79)	80/98, 81.63% (72.53-88.74)	.66

TABLE 1 Comparative results between dogs that received leukoreduced (LR) vs nonleukoreduced (N-LR) packed red blood cell (PRBC) transfusion between August 2017 and June 2020

Abbreviations: CI, confidence interval; FNHTR, febrile non hemolytic transfusion reactions; IQR, interquartile range; LR, leukoreduction; N-LR, nonleukoreduction; PRBC, packed red blood cells.

transfusion. For 5/15 dogs, the transfusion was stopped for a median of 30 minutes (IQR 52.5 minutes) and was then continued when their temperature normalized. In 9/15 cases, the transfusion was not interrupted. The incidence of FNHTRs was not significantly different between dogs that received LR (6/96, 6.25%, 95% CI 2.8-13.56) vs N-LR (9/98, 9.18%, 95% CI 4.92-17.11) PRBC ($P = .81$).

One dog (1/194, 0.51%) had a mild allergic reaction observed approximately 1 hours after the transfusion finished, characterized by mild facial swelling. A dose of 0.3 mg/kg of chlorphenamine was administered via intravenous injection and no other treatments were required.

One 2.29 kg dog (1/194, 0.51%) became hypothermic, with a change in temperature from 38.1 to 35°C 3 hours after the start of transfusion. The transfusion rate was decreased from 2 mL per kilogram per hour to 1 mL per kilogram per hour and external warming was provided.

Five (5/194, 2.57%) vomited during PRBC transfusion. The transfusion was stopped in 3/5 cases (for differing times of 5, 10, and 30 minutes) and maropitant was given in 2/5 dogs. None of the dogs that vomited during the transfusion presented with vomiting as a clinical sign and none of them had vomiting reported before or in the 24 hours following the blood transfusion. Two of the dogs that vomited during the PRBC transfusion were diagnosed with IMHA and 3 were diagnosed with IMTP. One of the dogs diagnosed with IMTP that vomited during the transfusion had severe hematemesis and persistent gastrointestinal signs (melena) reported.

One dog became tachypnoeic during the transfusion with an increase in the respiratory rate from 28 breaths per minute to 76 breaths per minute 3 hours after initiation of the transfusion. The transfusion was stopped, and the left atrium: aorta was checked and was found to be <1.5. The transfusion was restarted after 30 minutes and there were no further episodes of tachypnoea noted.

$APPLE_{FAST}$ scores were calculated for 159/194 (81.95%) dogs and the mean value was 24.79 (SD 5.49) for all cases, 25.27 (SD 5.73) for dogs that received LR PRBCs and 24.48 (SD 5.21) for dogs that received N-LR PRBCs. There was no significant difference in the mean $APPLE_{FAST}$ score value between the 2 groups ($P = .26$). The effect size was 0.18 (95% CI −0.10 to 0.46).

One hundred fifty-six (156/194, 79.38%) dogs survived to discharge after a median of 5 days of hospitalization (IQR 4 days), out of which a median of 2 days (IQR 2 days) were spent in the hospital's intensive care unit. Out of the dogs that survived to discharge, dogs that received LR PRBC were hospitalized for a median of 6 days (IQR 4 days), out of which a median of 2 days (IQR 2 days) were spent in the intensive care unit and dogs that received N-LR PRBC were hospitalized for a median of 5 days (IQR 4 days), out of which a median of 1 day (IQR 2.11 days) were spent in the intensive care unit. There was no significant difference in the median days of hospitalization between dogs that received LR vs N-LR PRBC ($P = .06$) or in the median days of ICU hospitalization between dogs that received LR vs N-LR PRBC ($P = .39$).

Out of the 38 dogs that did not survive to discharge, 33/38 (86.84%) were euthanized and 5/38 (13.15%) underwent cardiopulmonary arrest. Out of the 156 dogs that survived to discharge, 80 received N-LR PRBC and 76 received LR PRBC. There was no significant difference in survival to discharge between dogs that received LR vs N-LR PRBC ($P = .66$) (Table 1). There was also no association between survival to discharge and a dog having had a TR ($P = .12$).

Out of the 194 dogs, 96 received LR and 98 received N-LR PRBCs. The mean 12-hour change in PCV value was +9.22% (SD 5.27%) for dogs that received N-LR and +10.69% (SD 6.44%) for dogs that received LR PRBC, which was not significantly different ($P = .08$). TRs were documented in 16/194 (8.24%) dogs, with 1/194 (0.51%) being a mild allergic reaction, while 15/194 (7.73%) had suspected febrile nonhemolytic TRs (FNHTRs). FNHTR incidence was not significantly different between the LR (6/96, 6.25%, 95% CI 2.8-13.56) and N-LR (9/98, 9.18%, 95% CI 4.92-17.11) groups ($P = .81$). Of the 156 dogs that survived to discharge, 80/156 received N-LR PRBC and 76/156 received LR PRBC which was not significantly different ($P = .66$).

4 | DISCUSSION

This randomized prospective study explored the incidence of acute TRs in dogs receiving either LR or N-LR blood products and allowed us to examine the utility of leukoreduction in reducing morbidity. The results did not show a significant difference in the incidence of FNHTRs, change in PCV value after transfusion, days of hospitalization or survival to discharge between the 2 groups.

Theoretically, leukoreduction should be beneficial. Leukocytes present in stored blood can cause harm to the recipients via the presence of proinflammatory cytokines, by contributing to storage lesion, as a consequence of modulating the recipient's immune system or by transmission of viruses that exist within the leukocytes.[21] Leukocytes generate cytokines with pyrogenic activity in the stored blood, including IL-1, IL-6, IL-8, and TNF-α.[34] These proinflammatory cytokines play an important role in the incidence of TRs in human patients, especially FNHTR.[16] Prestorage leukoreduction reduces the incidence of FNHTRs in human medicine from 50% to 80%,[32,33,35,36] most likely by abrogating the accumulation of these cytokines.[10]

In vitro studies show that leukoreduction is effective in decreasing the levels of IL-6 and IL-8 in canine stored blood,[11,37,38] decreasing the presence of *Rickettsia conorii* in infected blood units,[39] preventing the release of vascular endothelial growth factor (VEGF) during storage[28] and decreasing procoagulant activity in stored PRBC.[29] In healthy dogs leukoreduction decreases the inflammatory response to transfusion of 21-day old PRBC,[40] measured by white blood cell and segmented neutrophil counts, fibrinogen concentration and C-reactive protein values. Another study, however, found no significant differences between LR and N-LR units in the inflammatory response induced by autologous blood transfusions, when measuring the white blood cell and absolute neutrophil counts.[41] In critically ill dogs that required blood transfusions, markers of inflammation are similar after transfusion of LR vs N-LR PRBC.[42] This latter study, along with the findings from this present study, suggests that although leukoreduction might play a role in decreasing inflammatory mediators in canine PRBC during storage and in reducing recipient inflammatory response, this might not necessarily be of clinical relevance. Moreover, a recent study of dogs in abstract form was not able to detect a reduction in the rate inflammatory response or in the rate of FNHTRs with leukoreduction (Poh D, Smart L, Purcell SL et al., *J Vet Emerg Crit Care, San Antonio*, 2019).

FNHTRs are the most common complication of PRBC transfusions in people[1,3,5] and dogs[43] and are usually ascribed to cytokines released by leucocytes and platelets during storage.[10]

The incidence of FNHTRs in people receiving PRBCs varies between 0.08% and 1.56%[7,35,44,45] but it is reportedly higher in dogs, varying between 3% and 24.17%.[24,25,43,46] Consistent with this, the most common TR reported in this study were FNHTRs, with an incidence of 7.73%.

In people, the incidence of FNHTRs is significantly higher for patients with no transfusion history[47] compared to patients that have received previous transfusions. While most human studies include patients that have received multiple transfusions, current veterinary literature mainly describes dogs that have received only 1 transfusion unit. This could be a contributing factor to the higher incidence of FNHTRs reported in veterinary medicine. Moreover, it is possible that mild undetected hemolytic reactions occur in veterinary medicine as a consequence of less robust blood typing and crossmatching practice when compared to human medicine. These reactions could be misclassified as FNHTRs due to less thorough investigations.

Leukoreduction has been shown to decrease the incidence of FNHTRs in human medicine,[10,48,49] especially in patients that have a history of previous FNHTRs.[21] FNHTRs, although common in human transfusion medicine, are not life-threatening,[49] and performing universal leukoreduction in human medicine in order to reduce their incidence is still debated.[20] Our study did not show a significant difference in the incidence of FNHTRs between the LR and N-LR groups ($P = .81$). Although leukoreduction has been showed to decrease the accumulation of proinflammatory cytokines in canine PRBCs,[11,37,38] it is possible that this effect is less important or that the accumulation of leukocyte derived cytokines play a less important role in the pathogenesis of FNHTRs in dogs compared to humans.

The detection of a FNHTR was not associated with survival to discharge or a variation in duration of hospitalization in our study. This is likely to be due to the fact that most FNHTRs are benign events, although they are associated with clinical signs such as chills, rigor, and discomfort in human patients[50] and with an increased workload and resource consumption in human hospitals.[51]

Vomiting was observed in 5/194 (2.57%) dogs during PRBC transfusion. Vomiting is a relatively common adverse event associated with canine blood product transfusion,[52] occurring in 4% of dogs receiving blood products.[24] Blood transfusions cause anaphylactic reactions in dogs,[52] and vomiting can be a sign of anaphylaxis, although this is usually suspected when 1 or more of: cutaneous signs, respiratory compromise, hypotension, and persistent vomiting or diarrhea occur after exposure.[53] Although the blood pressure was not monitored for these dogs, most of them did not have persistent vomiting or diarrhea reported, therefore anaphylaxis is unlikely, but vomiting can also be a sign of a milder allergic reaction.[24,54] Human literature includes nausea and vomiting in the FNHTR syndrome,[8] but none of the dogs that vomited during the transfusion had an associated increase in temperature in our study.

Only 1 dog had mild facial swelling suspected to be due to a mild acute allergic reaction. Acute allergic reactions characterized by

cutaneous manifestations such as urticaria are not commonly reported with PRBC administration in dogs,[43] as documented in our study.

During storage of PRBCs, a number of changes take place that can affect the viability and function of the red blood cells[13] and can contribute to an increased incidence of adverse events in human medicine.[55–59] Prestorage leukoreduction can reduce the formation of proinflammatory particles during storage in human and mice[16] and therefore can help reduce the incidence of storage related TRs. The age of the units was not associated with survival to discharge in our study and in contrast to the findings of a retrospective study looking into transfusion reactions in 558 dogs,[24] the presence of an immune mediated disease was not associated with an increased likelihood of acute transfusion reaction. There was no significant difference in the age of the PRBC unit received between dogs that had a FNHTR documented and dogs that did not have a FNHTR ($P = .67$).

Our study showed no difference in survival to discharge between the LR and N-LR groups. This finding is in agreement with most human medicine trials in which leukoreduction does not improve overall patient survival.[60,61] However, there is a significant reduction in deaths in human patients undergoing cardiac surgery receiving leukoreduced blood products[62,63] and 1 Canadian institution reported a significant reduction in deaths after implementing universal leukoreduction.[64]

Leukoreduction is a fairly straight forward procedure but purchasing leukoreduction filters increases the cost of the resulting blood products. Moreover, leukoreduction filtration results in a reduction of approximately 52 mL from the total volume of a standard canine whole blood unit.[26] Leukoreduction, therefore, might result in a lower amount of blood administered and some dogs might require additional units that increase the cost of treatment. Universal leukocyte reduction (ULR), defined as leukoreduction of all blood products in a country or in a blood transfusion service,[65] has a debatable superiority and cost-effectiveness in human medicine.[7,20,66,67]

A major limitation of this study is the lack of follow-up to assess for the presence of any delayed transfusion reactions (>24 hours after the end of the transfusion). Although delayed transfusion reactions are not commonly reported in dogs,[52] there is a possibility that they are significantly underreported and that they can affect the outcome of the animals. Moreover, we did not account for dogs that were receiving immunosuppressive treatment. One retrospective study looking at 210 dogs receiving blood transfusions found that dogs diagnosed with an immune mediated disease were more likely to develop transfusion reactions, and dogs receiving immunosuppressive therapy for IMHA were more likely to have acute transfusion reactions, especially febrile reactions.[43]

Additionally, the study design did not control for the age of the PRBCs, although there was no significant difference in the mean age of the units between LR and N-LR groups.

Despite designing this trial in accordance to a sample size calculation, it is still possible the study was underpowered to detect a significant difference in FNHTR rate between groups. A further limitation of our study is the lack of measurement of proinflammatory cytokines or other biomarkers that could have detected an inflammatory response within the N-LR, despite the lack of difference in clinical outcomes.

This study shows that, consistent with previously published literature, the incidence of acute transfusion reactions in dogs is relatively low and most acute transfusion reactions are FHNTRs, which are not life-threatening. Neither the incidence of FNHTRs nor survival to discharge were found to be affected by leukoreduction.

In this prospective randomized double-blinded clinical study, a clinical advantage of using LR PRBC over N-LR PRBC in terms of TRs and increase in PCV after transfusion could not be demonstrated.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approval granted by the Royal Veterinary College teaching hospital Ethics and Welfare committee (reference number URN M2015 0054).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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