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Evaluation of Stored Whole Blood and Monitoring the Health of Dogs After Transfusion Using Fresh Whole Blood, Stored Whole Blood, and Packed Red Blood Cells

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ABSTRACT

Blood products have been widely used in emergencies and treatment, necessitating optimal storage conditions to maintain quality. The current study aimed to evaluate the blood quality during storage, transfusion effectiveness, and reactions during and after transfusion in dogs. Five Greyhounds, including three males and two females aged 2.5 years old, and with 25-30 kg bodyweight, were selected and randomly labeled N1, N2, N3, N4, and N5. Fresh whole blood, stored whole blood, and packed red blood cells from the samples dogs were used for transfusion in the study. The investigated parameters were total protein (TP), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), mean corpuscular volume (MCV), total carbon dioxide (tCO_2), creatine kinase (CK), creatinine (CREA), blood urea nitrogen (BUN), glucose (GLU), white blood cells (WBC), red blood cells (RBC), hematocrit (HCT), plaletes (PLT), calcium (Ca), phosphorus (P), chloride (Cl), manganese (Mg), sodium (Na), and potassium (K). The results indicated that all parameters of stored blood samples were in the normal range during 28 days of storage in a refrigerator at 2-4°C. However, some parameters (TP, AST, ALT, ALP, LDH, MCV, tCO_2, and K) increased, while others (CK, CREA, BUN, GLU, WBC, RBC, HCT, PLT, Ca, P, Cl, Mg, and Na) decreased during the storage period, especially Ca, P, and Na were below the normal range. All dogs indicated no reactions during and 5 hours after transfusion. However, dogs had symptoms of inappetence and mild diarrhea in 1-2 days after transfusion. Dogs received fresh whole blood recovered on day 3, while dogs of the stored blood recipient group recovered on day 4. By day 5, all dogs were healthy with no abnormal signs. The findings indicated the presence of hematological and biochemical alterations in stored blood, highlighting the importance of considering transfusion of stored blood for patients with critical medical conditions.

Keywords: Dog, Fresh whole blood, Packed red blood cell, Stored whole blood, Transfusion

INTRODUCTION

Although the first time dogs were successfully transfused with blood was in 1665, blood transfusion in dogs and cats has become commonplace in over the last 60 years (Yagi and Holowaychuk, 2016a; Khan and Sharma, 2021). Blood products are widely used in emergencies and treatment. Transfusion is an effective therapy and is considered to be life-saving, which can be used in emergencies and can save dogs in severe anemia cases with a high risk of death (Hann et al., 2014). However, transfusion of red blood cell (RBC) concentrates might cause adverse effects in the recipient, particularly when stored for more than 2 weeks (Herring et al., 2013).

To ensure the utmost quality and safety of blood and blood products, the entire process must be carried out using a standardized system. The donors must be healthy, fully vaccinated, and carrying no infectious diseases (Yagi and Holowaychuk, 2016b). The blood collecting system and blood storage must be appropriately carried out. The hematology and serum biochemistry blood profile must be periodically tested. The hematological profile includes measurement of hemoglobin (HB), hematocrit (HCT), or packed cell volume (PCV), red cell indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]), total cell counts (RBC, platelets [PLT], and white blood cells [WBC]) differential white blood cells counts and comments by a veterinary hematologist on the morphology of the erythrocyte, leucocyte, and platelet populations (Day and Kohn, 2012).

Veterinarians performing the transfusion are crucial in guaranteeing the well-being of the patients. However, the limit in transfusion knowledge and experience of a practitioner could be a challenge. Furthermore, appropriate blood collection, processing, storage, and administration methods, which are usually not followed by practitioners correctly,

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can also be a problem. Any mistake in the whole transfusion process can cause serious side effects, be an infectious disease's route of transmission, or even lead to death (Moor et al., 1999; Goodnough, 2003; Buddeberg et al., 2008).

Blood storage must be carefully managed to ensure the appropriate storage duration and blood quality. Blood storage management is essential for the success and effectiveness of a treatment, and for avoiding wasting blood products (Bashir et al., 2021). Appropriate blood collection, processing, storage, administration methods, strict monitoring, and quick reaction to side effects are complicated and play an important role in blood transfusion (Dong and Vo, 2020). Veterinarians need to have enough knowledge of the transfusion process for treatment in emergency cases.

Increasing of anemia cases in dogs occurred with many causes, such as bloody diarrhea (caused by Parvovirus), blood parasites (*Ehrlichia canis, Anaplasma* spp., *Babesia canis*), endoparasites (*Toxocara canis, Ancylostoma caninum, Dipylidium caninum*), cancer, trauma (hit by a vehicle). The need for canine blood transfusion for these anemia cases is also increasing (Day and Kohn, 2012; Zakarevičiūtė et al., 2021). However, there are not many studies of blood storage and transfusion in veterinary hospitals and clinics in Vietnam. The current study was carried out to have a summary and evaluation of blood quality during storage, evaluation of transfusion effectiveness using fresh whole blood, stored whole blood and blood products, as well as the transfusion reactions during and after transfusion in dogs.

MATERIALS AND METHODS

Ethical approval

The present study was conducted by collecting blood samples and transfusion according to the procedures of the Animal Welfare Council, Nong Lam University, Ho Chi Minh City, Vietnam.

Experimental animals, blood collection, and storage

The study was conducted at Nong Lam University, Ho Chi Minh City, Vietnam. A total of five healthy Greyhounds, including 3 males and 2 females aged 2.5 years old, and weighing 25-30 kg were selected randomly and labeled N1, N2, N3, N4, and N5. All dogs were fully vaccinated within the last 12 months and received prophylactic flea treatment regularly. Dogs did not receive any raw meat diet and were completely healthy and relaxed. The N5 did not receive a blood transfusion from the others. The animals were fed with commercial dry feed (France) (900 gr). All dogs were checked physically and all clinical signs, including body temperature, heart and respiratory rate, and appetite were normal. An amount of 100 ml of blood was collected from each dog (Nong Lam Veterinary Hospital's blood bank protocol) and stored in commercially available blood bags (Ningbo Greetmed Medical Instruments Company), with anticoagulant–preservative solution (citric acid, sodium citrate, monobasic sodium sulfate, dextrose, and adenine) with 16-gauge needles. Before blood collection, the hair of the collection site was clipped. The dogs were calm and placed in lateral recumbency on a blanket, on a table. The jugular vein phlebotomy was conducted to fix the needle before collecting. The blood was collected into the blood bag containing 100 ml of blood. During the blood collection, all dogs were monitored for mucous membrane color, pulse rate, and respiratory rate. The collected blood was stored in a refrigerator at 2-4°C. The temperature of the refrigerator was checked and recorded every 12 hours with a thermometer. The whole stored blood of each dog was tested on hematological and biochemistry before performing a transfusion on days 0, 7, 14, and 28. In the present study, the IDEXX Procyte Dx® (IDEXX Laboratories Incorporation, United States) hematology analyzer was used and the IDEXX Catalyst One (IDEXX Laboratories Incorporation, United States) blood biochemistry analyzer was used as well. The hematology analyzer's work principles are laser flow cytometry, optical fluorescence, and laminar flow impedance. The commercial kits for blood biochemistry and ion tests were Chem 17 Clip and Lyte 4 CLIP, respectively (IDEXX Laboratories Incorporation, United States). The reference ranges of hematology and biochemistry parameters for adult canines were also from IDEXX Laboratories Inc., United States (IDEXX Catalyst, 2019; IDEXX ProCyte Dx, 2019).

The average of hematology and biochemistry parameters was calculated for comparison. The parameters were calculated, including white blood cells (WBC), red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), platelets [PLT], total protein (TP), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), creatinine (CREA), blood urea nitrogen (BUN), and glucose (GLU). The calculated ion parameters included (Ca), phosphate (Phos), total carbon dioxide (tCO_2), chloride (Cl), potassium (K), manganese (Mg), and sodium (Na).

Blood was transfused initially at day 0, and day 14 was the day of the second blood transfusion. The Greyhounds were divided into 2 groups, including the fresh whole-blood recipient group and the stored whole-blood recipient group. The N1 (day 0), N4 (day 0), N2 (day 14), and N3 (day 14) were in the fresh whole-blood group and the stored whole-blood group included N1 (day 14), N4 (day 14), N2 (day 0) and N3 (day 0).

Serologic compatibility tests

Before each transfusion, a compatibility test was performed to detect naturally occurring antibodies or all antibodies produced as a consequence of sensitization by glass slides under a light microscope (Model Optika IM-3MET, Italy). The compatibility test carried out in the study was crossmatching, including major crossmatch assessment and minor crossmatch assessment. The rapid slide method was applied at 37°C (Day and Kohn, 2012). These steps included collecting blood into an EDTA tube from the recipient and donor, then centrifuge tubes to settle the RBC and plasma. On each glass slide, place 1 drop of RBC and 2 drops of plasma, then mix with an applicator stick, there were four glass slides for each pair of donor and recipient dogs, labeled as donor control (donor RBC and donor plasma), major crossmatch (donor RBC and recipient plasma), minor crossmatch (recipient RBC and donor plasma) and recipient control (recipient RBC and recipient plasma). Finally, the macroscopic agglutination on the slides within 2 minutes and microscopic agglutination with oil immersion at x100 lens within 5 minutes were done. A positive result is determined by the appearance of an agglutination reaction (Day and Kohn, 2012).

Blood transfusion between dogs

Before the transfusion, each recipient was drawn 100 ml of blood. They were transfused to the same volume of blood, to ensure the total body fluid did not change. The N5 did not receive any blood from other dogs since it was used to produce pure red blood cells in the blood transfusion experiment. Before the initial transfusion, a total of 100 ml of blood was collected from each dog for storage, for 14 days. Stored whole blood was left at room temperature for approximately 30 minutes to warm up the blood and avoid hypothermia. The administrated route was intravenous. Initial blood was administered at the rate of 1 ml/kg/hour for the first 20 minutes to observe transfusion reactions, then increased to 2 ml/kg/hour (total of 130 minutes; Day and Kohn, 2012). Blood was transfused in approximately 2 hours for each recipient. The recipients were monitored during transfusion, 5 hours, and 4 days after transfusion for any reactions. To increase the viscosity of the stored whole blood and reduce the risk of embolism during transfusion, dogs could be transfused 40 ml of NaCl with blood.

Before the initial transfusion, stored whole blood was inspected for discoloration of red cells, the presence of clots, or hemolysis for safety. In the first transfusion, N1 and N4 received fresh whole blood from N2 and N3, respectively. The N2 and N3 received stored whole blood from N5 and N1, respectively (blood had been stored for 14 days). In the second transfusion on day 14, all recipients received blood from N5, including N1 and N4 received stored whole blood (the blood had been stored for 28 days), and N2 and N3 received fresh whole blood.

Monitoring

Before transfusion, both hematological and biochemistry tests were conducted on the blood to ensure its suitability. These tests were performed regardless of whether the blood was freshly collected from the donor or stored as whole blood. The physical examination (body temperature, mucosa color, heartbeat, and respiratory rate) of each recipient was recorded by veterinarians. After the transfusion, the recipients were monitored on the day they received blood (day 0 and day 14) and 4 days later. On the day of transfusion, 5 hours after transfusion the recipients were checked every 1 hour. The symptoms monitored on the transfusion day were fever, hemorrhage, cyanosis, polynea/dyspnea, urticaria/angioedema, vomiting, and diarrhea. In the next 4 days, the recipients were monitored for body temperature, gums' color, heartbeat, respiratory rate, appetite, diarrhea, and vomiting by clinical observation. The recipient dogs' blood was then checked again 14 days after transfusion.

Statistical analysis

Data were analyzed by an average mean of the values through Microsoft Excel, version 2016.

RESULTS

Evaluation of fresh whole blood and stored whole blood during storage

The average of each parameter of fresh whole blood and stored whole blood is presented in Table 1. The reference ranges are from IDEXX (IDEXX Laboratories, Incorporation, United States). All fresh whole blood parameters from 5 dogs were in the normal range. The stored blood in the present study had some changes, but all parameters were still in the normal range on day 28 of storage, which made the stored whole blood available for transfusion. The PLT significantly decreased from day 2, while WBC gradually decreased in the storage period. In the current study, there was a decrease in both RBC and HCT. The average value of WBC and PLT markedly decreased in the storage period, while the mean corpuscular volume MCV increased. Serum biochemical analysis results in the present study indicated that there was an increase in TP, AST, ALT, ALP, LDH, and a decrease in CREA, BUN, and GLU. The increase of

parameters, including TP, AST, ALT, ALP, LDH, MCV, tCO_2, K, and the decrease of the other parameters, including CK, CREA, BUN, glucose GLU, WBC, RBC, HCT, PLT, Ca, P, Cl, Mg, and Na was observed during the storage period, with Ca, P, and Na were below the normal range (Tables 2, 3).

Although changes were observed in serum biochemistry results, all parameters were still in the normal range on day 28 of storage, except for a slight increase in AST. Meanwhile, ion parameters changed during storage, and most ion parameters were out of the normal range on day 28 of storage. Only Mg and Phos results were normal. The ion results of the present study also indicated an increase in K and LDH, and a decrease in Na level. The Na level dropped specifically below the normal range on day 7 of storage (138.4 mmol/L) and kept decreasing to 114.04 mmol/L on day 28.

Table 1. Th effects of blood collection day and storage period on hematology parameters

Parameter	Unit	Reference range*	Day 0	Day 7	Day 14	Day 21	Day 28
WBC	10^9/L	5.05-16.76	13.228	10.8	9.188	7	4.624
RBC	10^{12}/L	5.65-8.87	6.574	6.374	6.056	5.744	5.384
HCT	%	37.3-61.7	45.34	44.38	43.6	44	40.6
MCV	fL	61.6-73.5	70.48	71.34	73.2	74	75.22
PLT	10^9/L	148-484	216.6	171	146	119	98.4

WBC: White blood cells; RBC: Red blood cells; HCT: Hematocrit; MCV: Mean corpuscular volume; PLT: Plaletes; *The reference range of adult canine was from IDEXX Laboratories Inc., United States

Table 2. Th effects of blood collection day and storage period on biochemistry parameters

Parameter	Unit	Reference range*	Day 0	Day 7	Day 14	Day 21	Day 28
TP	g/L	52-82	67.74	70.76	72.4	75.28	77.5
AST	U/L	0-50	28.4	31.4	38	44.6	53.8
ALT	U/L	10-125	65.4	50	56	63	65
ALP	U/L	23-212	93.6	95	102.4	111.8	119.8
LDH	U/L	40-400	65	95	156	173	212
CK	U/L	10-200	107.6	112.4	99.8	93.6	87
CREA	mmol/L	44-159	61.84	45.12	46.38	47.41	45.26
BUN	mmol/L	2.5-9.6	5.352	5.23	4.28	4.3	4.21
GLU	mmol/L	4.11-7.94	5.098	5.446	5.222	4.982	4.446

TP: Total protein; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase; CK: Creatin kinase; CREA: Creatinine; BUN: Blood urea nitrogen; GLU: Glucose; *The reference range of adult canine was from IDEXX Laboratories Inc., United States

Table 3. Th effects of blood collection day and storage period on ion concentrations

Parameter	Unit	Reference range*	Day 0	Day 7	Day 14	Day 21	Day 28
Ca	mmol/L	1.98-3.00	2.392	2.236	2.076	1.674	1.46
Phos	mmol/L	0.81-2.19	1.472	1.34	1.242	1.112	0.986
tCO_2	mmol/L	21-31	17	17.42	17.82	18.32	18.72
Cl	mmol/L	109-122	104.54	110.4	120.2	107.74	102.664
K	mmol/L	3.5-5.8	4.764	5.074	5.436	5.642	5.98
Mg	mmol/L	0.58-0.99	0.912	0.868	0.832	0.77	0.692
Na	mmol/L	144-160	145.3	138.4	130.88	124.18	114.04

Ca: Calcium; Phos: Phosphate; tCO2: Total carbon dioxide; Cl: Chloride; K: Potassium; Mg: Manganese; Na: Sodium; *The reference range of adult canine was from IDEXX Laboratories Inc., United States

Symptoms of recipients on transfusion day and 4 days after

There were no positive results in compatibility tests between each pair of donor and recipient. In the present study, the body temperature, mucosa color, heartbeat, and respiratory rate of recipient dogs were recorded. There were no signs of transfusion reactions in any of the dogs five hours post-transfusion. All dogs in the study had no reactions during and 5 hours after transfusion. The average body temperature showed that stored blood recipients' body temperature was slightly higher than the fresh whole blood. The average body temperature of fresh whole blood recipients was 38.8°C,

while the stored whole blood recipients were 39.2°C. During 4 days after transfusion, the average body temperature of fresh whole blood recipients and stored whole blood recipients was 38.4°C and 38.75°C, respectively. The average heartbeat was 125 beats per minute and respiratory rate was 18.5 breaths per minute (fresh whole blood recipients) and 20 breaths per minute (stored whole blood recipients). All recipients had normal mucosa color (pink). All dogs had symptoms of inappetence and mild diarrhea in 1-2 days post-transfusion. In the fresh whole blood recipient group, all recipients recovered on day 3. In the stored blood recipient group, recipient dogs slowly recovered on day 4. Eventually, on day 5, all dogs were healthy with no abnormal signs. Dogs that received stored whole blood suffered from mild diarrhea longer than ones that received fresh whole blood. All recipients in both groups did not have any abnormal body temperature, heartbeat, respiratory rate, or mucosa color 4 days after transfusion. The recipients of stored whole blood (14 days and 28 days of storage) suffered post-transfusion reactions (inappetence and diarrhea) longer than the recipients of fresh whole blood one day.

Evaluation of recipients' blood after 14 days of transfusion

The average values of hematology parameters between the fresh whole-blood recipient group and the stored whole-blood recipient group were not significantly different (Table 4). In both groups, the average values of WBC and RBC were out of the normal range, while the others were in the normal range. The maximum RBC value of stored whole blood was still below the normal range (5.60×10^{12}/L). The average values of biochemical parameters were all in the normal range in both groups (fresh whole-blood and stored whole-blood recipient group; Table 5). In the ion test, there was almost no difference between these two groups. However, the average value of total carbon dioxide and chloride were lower compared to the normal range (Table 6).

In the current study, recipients of stored whole blood suffered side effects longer than recipients of fresh whole blood. Side effects did not vary between recipients of whole blood stored for 14 days and those stored for 28 days within the first two weeks post-transfusion. Although all recipients were healthy and alive, they still had mild transfusion reactions 2-4 days after transfusion.

Table 4. Average of hematology parameters of Greyhounds dogs aged 2.5 years on day 14 after transfusion

Parameter	Unit	Reference range*	Fresh whole blood			Stored whole blood		
			Avg. value	Min. value	Max. value	Avg. value	Min. value	Max. value
WBC	10^9/L	5.05-16.76	17.87	17.21	18.46	17.59	16.46	18.46
RBC	10^{12}/L	5.65-8.87	5.35	4.90	5.90	5.32	4.90	5.60
HCT	%	37.3-61.7	40.15	38.60	43.00	40.60	38.60	42.78
MCV	fL	61.6-73.5	69.55	66.80	72.10	70.23	66.80	74.20
PLT	10^9/L	148-484	207.50	121.00	248.00	187.25	121.00	259.00

WBC: White blood cells; RBC: Red blood cells; HCT: Hematocrit; MCV: Mean corpuscular volume; PLT: Plaletes; Avg. value: Average value; Min. value: Minimum value; Max. value: Maximum value; *The reference range of adult canine was from IDEXX Laboratories Inc., United States

Table 5. Average of biochemical parameters of Greyhounds dogs aged 2.5 years on day 14 after transfusion

Parameter	Unit	Reference range*	Fresh whole blood			Stored whole blood		
			Avg. value	Min. value	Max. value	Avg. value	Min. value	Max. value
TP	g/L	52-82	74.60	64.80	81.10	74.70	63.90	91.60
AST	U/L	0-50	36.00	15.00	54.00	34.00	22.00	45.00
ALT	U/L	10-125	78.00	35.00	132.00	79.75	35.00	145.00
ALP	U/L	23-212	81.00	27.00	160.00	80.75	36.00	160.00
LDH	U/L	40-400	171.75	65.00	243.00	162.50	65.00	231.00
CK	U/L	10-200	89.00	39.00	174.00	90.50	29.00	164.00
CREA	mmol/L	44-159	112.80	56.30	169.30	116.23	68.60	157.00
BUN	mmol/L	2.5-9.6	7.88	6.40	9.70	8.18	6.82	9.70
GLU	mmol/L	4.11-7.94	5.32	4.26	6.47	5.75	4.32	6.91

TP: Total protein; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase; CK: Creatin kinase; CREA: Creatinine; BUN: Blood urea nitrogen; GLU: Glucose; Avg. value: Average value; Min. value: Minimum value; Max. value: Maximum value; *The reference range of adult canine was from IDEXX Laboratories Inc., United States

Table 6. Average ion concentrations of Greyhounds dogs aged 2.5 years on day 14 after transfusion

Parameter	Unit	Reference range*	Fresh whole blood			Stored whole blood		
			Avg. value	Min. value	Max. value	Avg. value	Min. value	Max. value
Ca	mmol/L	1.98-3.00	2.07	1.94	2.32	2.07	1.94	2.14
Phos	mmol/L	0.81-2.19	1.23	0.57	1.87	1.24	0.57	2.01
tCO_2	mmol/L	21-31	20.50	15.70	25.60	19.35	15.30	25.60
Cl	mmol/L	109-122	86.45	78.40	96.40	88.65	76.50	99.30
K	mmol/L	3.5-5.8	4.32	3.90	4.99	4.02	2.90	5.01
Mg	mmol/L	0.58-0.99	0.77	0.73	0.84	0.76	0.67	0.88
Na	mmol/L	144-160	129.08	108.20	144.80	132.38	112.70	144.80

Ca: Calcium; Phos: Phosphate; tCO2: Total carbon dioxide; Cl: Chloride; K: Potassium; Mg: Manganese; Na: Sodium; Avg. value: Average value; Min. value: Minimum value; Max. value: Maximum value; *The reference range of adult canine was from IDEXX Laboratories Inc., United States

DISCUSSION

Stored whole blood changes

There were no abnormal findings (discoloration of red cells, hemolysis, or blood clots) in visual inspection of the stored whole blood and inspection of the blood bags before transfusion. In human research, stored whole blood samples after 35 days had significant cellular changes, such as WBC, RBC, and PLT decrease, while MCV and HCT increased (Maruti et al., 2021). In a study by Al Nuaimy (2008), Hemoglobin (HGB) and PCV significantly decreased after ten days of storage in humans. It was unlikely that there was potassium accumulation in canine and feline, except for some Japanese and Korean dog breeds which had high RBC concentrations of K (Obrador et al., 2015). Stored whole blood in humans had increased K and LDH levels, and Na and pH levels significantly decreased (Oyet et al., 2018; Marabi et al., 2020).

Observation of recipients' reactions after transfusion

Transfusion reactions were probably under-recognized but were reported to occur in 3.3-28% of dogs and 1.2-8.7% of cats (Hohenhaus, 2010). During transfusion, monitoring plays an important role in blood transfusion. The parameters should be measured which were attitude behavior, rectal temperature, pulse rate and quality, respiratory rate and character, mucus membrane color, and capillary refill time (Day and Kohn, 2012). A transfusion reaction was most likely to occur within the first hour of the patient receiving blood (Mullineaux and Jones, 2007). Although the study was conducted with healthy individuals, dogs still had transfusion reactions for at least 3 days, which could be a major problem in patients with more severe conditions.

Evaluation of recipients' blood after 14 days of transfusion

A study of 3,095 dogs indicated that the duration of packed red blood cell (PRBC) storage did not appear to be a major contributing factor to mortality in the overall canine population, however, the longer duration of PRBC storage might negatively impact the outcome in dogs with immune-mediated hemolytic anemia (Hann et al., 2014). A mild increase in WBC, a decrease in RBC, total CO2, and chloride in both groups were observed. The selected dogs in the study were deemed to be in good health, potentially contributing to the slight alteration in blood cells, in addition to close monitoring. Accordingly, of dogs with hemolysis, 90% of which had immune-mediated hemolytic anemia, a longer duration of PRBC storage was a negative risk factor for survival (Hann et al., 2014).

Serum biochemistry changes altered the RBC and membrane structure, resulting in compromised microvascular blood flow after transfusion and increased hemolysis during storage, respectively (Barshtein et al., 2011). Some RBC changes were immediate, such as a decrease in 2.3-diphosphoglycerate (2.3-DPG), whereas alterations in lactate, pH, or adenosine triphosphate (ATP) occurred over days (Cohen and Matot, 2013). These events included alterations in the cell membrane that might lead to the formation of microparticles (MPs) which may increase from a variety of cell types under normal physiologic conditions, including RBCs, platelets, endothelial cells, and WBCs (Herring et al., 2013). The change in lactate dehydrogenase over time, which could also affect the pH of the stored blood, was also observed (Herring et al., 2013). The occurrence of platelet storage lesions was well recognized and further contributed to the short storage time. Changes in pH, LDH concentration, and morphology changes, could alter the efficacy of the platelets when transfused (Callan et al., 2009; Devine and Serrano, 2010). Not only platelets but also all hematology parameters had a

decrease below the normal range on day 28 of storage in the study (except HCT). Biochemical, biomechanical, and immunologic changes during the storage of whole blood could affect red cell viability, deformability, oxygen-carrying capacity, microcirculatory flow, and recipient response (Obrador et al., 2015).

CONCLUSION

The stored blood had some changes of composition during 28 days of storage, but all parameters of hematological and biochemistry results were in the normal range, and available for blood transfusion in adult Greyhounds. All the dogs that received the transfusion experienced a loss of appetite and mild diarrhea within 1-2 days. However, they fully recovered and displayed no abnormal symptoms after the 5-day transfusion period. The hematology parameters between the fresh whole blood recipient group and stored whole blood recipient group did not differ from each other after a 14-day transfusion, so both fresh whole blood and stored whole blood would become blood sources for transfusion in dogs. According to the obtained results, both hematological and biochemical changes in canine blood during storage, and stored blood transfusion could cause side effects for recipients with severe conditions. The study of blood transfusion needs to be conducted in dogs of different breeds and ages and evaluate the effects of blood transfusion in disease cases.

DECLARATIONS

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Thuong Thi Nguyen, Hoa Thi Quynh Nguyen conceptualized, designed, and supervised the research. Thuong Thi Nguyen, Hoa Thi Quynh Nguyen collected samples and conducted experiments. Thuong Thi Nguyen, Khanh Nguyen Dinh analyzed and interpreted the data generated. Thuong Thi Nguyen, Hoa Thi Quynh Nguyen, Khanh Nguyen Dinh critically reviewed the study. All authors revised and approved the final manuscript.

Availability of data and materials

The authors of this article confirm that all data supporting the findings of this research are available upon reasonable request from authors.

Ethical considerations

The authors considered ethical concerns and consent, animal welfare and safety procedures before conducting the study. This article was written originally without any copy from data of published articles and books.

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