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Original Research Article

Effects of CPDA-1, ACD and Heparin Anticoagulants on the Quality of Canine Whole Blood during Storage

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Abstract: This study was conducted to compare the effects of three common anticoagulants (CPDA-1, ACD, and Heparin) on the quality of canine whole blood during 21 days of storage at 4 °C. A total of 90 blood samples from eligible dog donors were randomly divided into three experimental treatments using anticoagulants CPDA-1, ACD and Heparin, respectively. Hematological parameters were periodically analyzed on days 0, 1, 3, 7, 14, and 21. The results showed that CPDA-1 was the superior anticoagulant, best maintaining the stability of red blood cell parameters (RBC, HGB, HCT, MCV) while significantly slowing the decline in white blood cell (WBC) and platelet (PLT) counts. In contrast, Heparin demonstrated the poorest preservation efficacy, causing a severe deterioration in red blood cell quality and an almost complete loss of platelet function; while ACD yield intermediate results. These changes are characteristic manifestations of "storage lesions." Therefore, this study confirms that CPDA-1 is the primary choice for the long-term storage of canine blood in veterinary blood banking practice.

Keywords: ACD, Anticoagulant, Blood preservation, Canine, CPDA-1, Heparin.

1. INTRODUCTION

Blood transfusion is an essential treatment in veterinary medicine, especially in canine emergencies such as Parvovirus, Ehrlichiosis, Babesiosis or acute blood loss due to trauma and surgery. The demand for blood for dogs in major veterinary hospitals around the world increases steadily by 5–8% each year; of which, whole blood accounts for about 35–45% of indications in severe cases (Davidow *et al*., 2022). In Vietnam, a similar increasing trend has been recorded in urban areas and areas with high pet density, typically the Central region (Thuy, 2018).

However, whole blood storage for dogs still faces many challenges: quality deterioration over time, risk of bacterial contamination during storage, and especially the lack of standardized procedures suitable for practical conditions. Recent evidence shows that the shelf life of whole blood in dogs ranges from 21–35 days, depending on the type of anticoagulant and cold storage conditions. CPDA-1 is currently widely used because of its adenine component that helps maintain red blood cell ATP, thereby stabilizing hematological parameters for 28–35 days (Taylor *et al*., 2023); in contrast, Heparin is less recommended because of the high risk of hemolysis (Davidow *et al*., 2022). In addition to the choice of anticoagulant, maintaining a temperature of 2–6 °C and controlling bacterial contamination play a role in determining the quality of blood units (AbramsOgg and Blois, 2022).

In Vietnam, research data is still limited, with ACD, the actual storage time is usually only 14–21 days; the hemolysis rate increases significantly after 7 days when cold storage and sterile conditions are not optimal (Thanh, 2019). In the context of increasing demand for blood transfusion but lacking standard storage procedures, the rate of transfusion failure (15–18%) and adverse reactions such as fever and hemolysis remain at a worrying level (Thuy, 2018). The large gap in data on hematological characteristics over storage time, as well as the effectiveness of anticoagulants in actual clinical conditions in the Central region, is hindering the establishment of a professional veterinary blood bank. Based on that reality, the study was conducted to provide a scientific basis for optimizing blood storage procedures, improving efficiency and safety in treatment.

2. MATERIALS AND METHODS

2.1. Materials and Equipment

The study was conducted from June 2025 to October 2025 on 90 blood samples collected from 90 healthy dog donors. Individuals were selected based on strict criteria: age 1–7, weight 15–20 kg, clinically

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healthy, negative for infectious diseases and bloodborne parasites, no history of blood transfusion and no use of antibiotics within 4 weeks before sampling.

The study was designed as a randomized controlled trial. Ninety dogs were randomly assigned to three groups (n=30 each), corresponding to the three types of anticoagulants used: group 1 (CPDA-1) - blood was mixed with CPDA-1 solution at a ratio of 1:7; group 2 (ACD) - blood was mixed with ACD solution at a ratio of 1:6 and group 3 (Heparin) - blood was treated with Heparin at a concentration of 10 IU/mL.

The anticoagulants used included Citrate Phosphate Dextrose Adenine (CPDA-1) and Acid Citrate Dextrose (ACD) solutions (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and Heparin (Sanofi, Paris, France). Hematological indices were analyzed using a BC-2800Vet automated hematology analyzer (Mindray, Shenzhen, China).

2.2. Sample Collection, Processing and Preservation Procedures

Blood was collected from the jugular vein under sterile conditions using an 18G needle and syringe. Immediately after collection, the blood was transferred to bags containing the corresponding anticoagulant and mixed gently to ensure complete dissolution. The blood bags were labeled with the code number, date of collection and type of anticoagulant. All samples were stored in a medical refrigerator at 2–6°C, 60–70% humidity, protected from vibration and direct light, following the recommendations of Wardrop and Birkenheuer (2023).

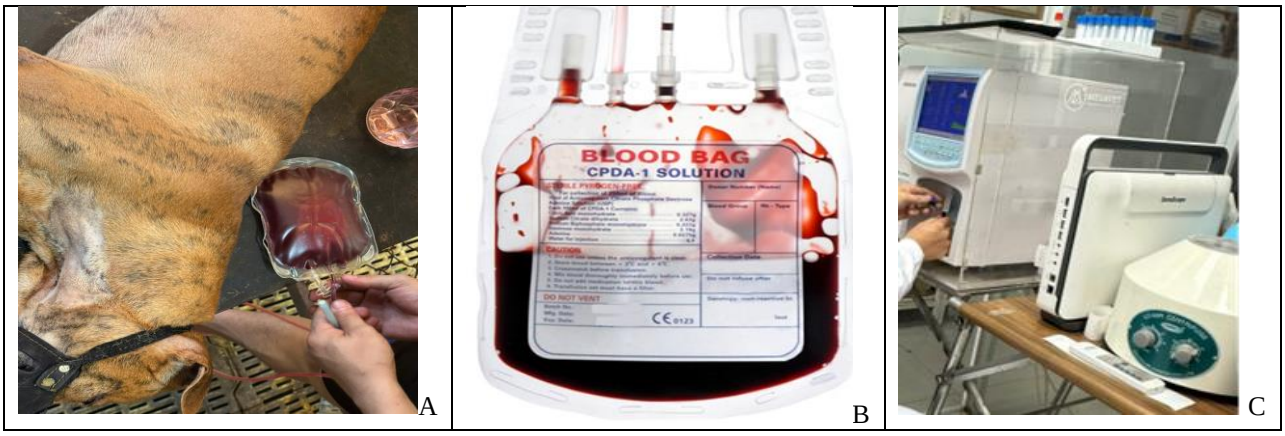

Figure 1: Blood sample collection (A), preservative (B) and hematological analysis (C).

2.3. Research Indicators

Hematological parameters were assessed at the following time points: day 0 (immediately after sampling), 1, 3, 7, 14 and 21 of storage. Parameters analyzed included: hematocrit (HCT, %), hemoglobin concentration (HGB, g/dL), red blood cell count (RBC, 10^6/µL), white blood cell count (WBC, 10^3/µL), platelet count (PLT, 10^3/µL), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dL). Instrument operating procedures and internal quality control were performed consistently for all samples analyzed.

2.5. Statistical Analysis

Data were processed using SPSS version 20.0 software. One-way ANOVA combined with LSD post-hoc test was used to compare the differences in mean values of indicators between groups at each time point and the changes over time within the same group; the difference was considered statistically significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Changes in Red Blood Cell Indices

3.1.1. Changes in RBC, HGB and HCT indices

The study's results indicate that all red blood cell parameters (RBC, HGB, HCT) progressively declined over the 21-day storage period; however, the rate and extent of this deterioration were significantly dependent on the anticoagulant used. This phenomenon, known as storage lesion, encompasses a series of biochemical and biomechanical changes that affect erythrocyte viability and function (Hess and Thomas, 2017).

Blood preserved with CPDA-1 demonstrated superior stability. The HGB and HCT indices remained stable with no statistically significant changes throughout the entire study period, while the RBC count only showed a significant decrease ($p < 0.05$) at the final time point on day 21. This outstanding preservation capacity is primarily attributed to the adenine component in the CPDA-1 solution. Adenine serves as a crucial substrate for ATP resynthesis via the salvage pathway, providing the necessary energy to maintain erythrocyte membrane integrity, ion pump function, and overall cell

viability (Taylor *et al*., 2023). This finding is consistent with previous research highlighting that adenine supplementation is key to extending the shelf-life of stored red blood cells to 35 days (Hess, 2010).

In stark contrast, the Heparin group exhibited the poorest preservation efficacy. All three indices—RBC, HGB, and HCT—decreased sharply and with high statistical significance ($p < 0.05$) starting from day 14. This outcome was anticipated, as heparin functions solely as an anticoagulant by potentiating antithrombin III and does not contain any nutritional components like dextrose or membrane-stabilizing additives like adenine (Weingart and Kohn, 2012). Consequently, erythrocytes stored in heparin rapidly deplete their energy reserves, leading to increased hemolysis and oxidative damage, reinforcing the consensus that heparin is unsuitable for long-term blood storage for transfusion purposes (Davidow *et al*., 2022; Swan *et al*., 2016).

The ACD group yielded intermediate results, proving more effective than Heparin but markedly inferior to CPDA-1. A significant decrease in HGB concentration was observed from day 7 onward. This can be explained by ACD's composition: it contains dextrose to support glycolysis but lacks adenine, thereby limiting the cell's capacity for effective ATP regeneration. This limited preservation window is consistent with findings from Thanh (2019) under similar conditions in Vietnam.

From a clinical application standpoint, these findings provide clear evidence that CPDA-1 should be the anticoagulant of choice for veterinary blood banking. Its ability to preserve the structural and functional integrity of erythrocytes is paramount for ensuring the quality, safety, and therapeutic efficacy of transfused blood, especially when storage exceeding two weeks is required.

Table 1: Changes in red blood cell indices RBC, HGB and HCT over storage time

Storage time (days)	RBC (10^6/mm^3)			HGB (g/dL)			HCT (%)		
	CPDA-1	ACD	Heparin	CPDA-1	ACD	Heparin	CPDA-1	ACD	Heparin
0	6.78^{a} ±0.34	6.72±0.40	6.82^{a} ±0.73	14.81±1.18	16.02^{a} ±1.37	15.04^{a} ± 0.92	44.99±4.3	45.07±4.31	45.69^{a} ±4.07
1	6.71^{a} ± $^{0.71}$	6.69±0.38	6.84^{a} ±0.74	14.75±1.20	15.40^{ab} ±1.33	14.96^{a} ±0.89	44.87±4.2	43.45±4.07	45.47^{a} ±3.92
3	6.64^{a} ±0.43	6.64±0.38	6.74^{a} ±0.71	14.71±1.19	15.18^{ab} ±1.23	14.75^{ab} ±0.86	44.63±4.2	42.89±2.47	44.92^{a} ±3.75
7	6.53^{ab} ±0.75	6.57±0.37	6.60^{ab} ±0.70	14.55±1.15	14.47^{b} ±1.08	14.43^{ab} ±0.84	44.38±4.3	43.46±2.78	43.83^{a} ±3.80
14	6.56^{ab} ±0.58	6.42±0.35	6.30^{ab} ±6.79	14.22±1.20	14.47^{b} ±1.02	13.84^{b} ±0.87	43.18±4.2	43.42±2.74	41.46^{ab} ±3.59
21	6.4 5^{b} ±0.50	6.29±0.39	6.04^{b} ±0.64	13.99±1.14	14.40^{b} ±1.02	13.15^{b} ±0.81	41.89±4.1	41.36±4.19	39.14^{b} ±3.02

***Note**:* In the same column, values with different superscript letters (a,b) indicate statistically significant differences ($p < 0.05$)

3.1.2. Changes in MCV, MCH and MCHC Indices

The study revealed that the most significant morphological change during storage occurred in the mean corpuscular volume (MCV), a critical indicator of erythrocyte membrane integrity and osmotic balance. In the CPDA-1 group, the MCV remained remarkably stable, showing no statistically significant change over the 21-day period. This superior stability is directly attributed to the optimal composition of the CPDA-1 solution. The presence of both dextrose and adenine ensures sustained ATP synthesis; ATP is essential for maintaining the function of transmembrane ion pumps, such as the Na+/K+-ATPase, which regulate intracellular hydration and preserve normal cell volume and deformability (Hess, 2010).

Conversely, both the ACD and Heparin groups exhibited a progressive and significant decrease in MCV, a classic manifestation of the "storage lesion" where erythrocytes undergo shrinkage (D'Alessandro *et al*., 2015). The decline was most rapid and severe in the Heparin group ($p < 0.05$ after only 7 days). This was expected, as heparin acts purely as an anticoagulant and provides no metabolic substrates. Without an energy source, erythrocytes rapidly deplete their ATP stores, leading to ion pump failure, subsequent dehydration, and membrane damage (Scott *et al*., 2005). The ACD group also recorded a statistically significant decrease in MCV by day 21 ($p < 0.05$). Although ACD contains dextrose to fuel glycolysis, the lack of adenine severely limits ATP regeneration via the salvage pathway, resulting in a gradual failure of osmotic regulation and subsequent cell shrinkage.

It is noteworthy that the indices related to hemoglobin content, MCH and MCHC, remained relatively stable across all three groups. This suggests that the initial phase of storage-induced damage primarily affects the cell membrane's ability to regulate volume, rather than causing a significant loss of intracellular hemoglobin. Massive hemoglobin leakage, a sign of advanced hemolysis, typically occurs at later stages of the degradation process (D'Alessandro *et al*., 2015).

Thus, the change in MCV serves as a sensitive and early biomarker for the deterioration of red blood cell quality. These results confirm the pivotal role of anticoagulant selection in preserving the morphological integrity of erythrocytes during storage, with CPDA-1 being the optimal choice for this purpose.

Table 2: Changes in red blood cell indices MCV, MHC and MCHC over storage time

Storage time (days)	MCV (fL)			MHC (pg)			MCHC (g/dL)		
	CPDA-1	ACD	Heparin	CPDA-1	ACD	Heparin	CPDA-1	ACD	Heparin
0	69.87±4.46	69.92^{a} ±1.96	73.15^{a} ±2.07	22.87±2.55	21.79±1.00	22.84±1.26	33.66±0.93	34.47±0.99	35.12±1.31
1	69.11±4.18	69.57^{a} ±3.92	72.21^{a} ±2.19	22.53±1.33	22.40±2.25	22.78±1.75	33.35±0.73	33.67±1.08	34.16±1.14
3	69.95±3.21	68.71^{a} ±2.88	69.37^{ab} ±2.21	22.24±0.75	21.84±1.61	22.31±1.87	33.61±0.64	33.42±0.74	33.62±0.66
7	68.38±2.33	67.93^{a} ±5.24	67.36^{b} ±2.00	22.33±1.61	21.98±1.95	22.34±1.92	32.91±1.13	33.23±0.46	33.23±0.46
14	68.44±2.47	66.16^{ab} ±2.34	69.87^{ab} ±4.46	21.85±0.40	21.47±1.87	21.70±0.43	32.59±0.37	33.63±0.88	33.63±0.88
21	67.56±1.86	64.45^{b} ±3.87	67.44^{b} ±3.10	22.22±1.21	20.72±0.99	21.75±1.67	32.90±0.41	33.49±0.40	33.36±0.64

***Note**:* In the same column, values with different superscript letters (a,b) indicate statistically significant differences ($p < 0.05$)

3.2. Changes in White Blood Cell and Platelet Indices

Refrigerated storage induced significant degradation of white blood cells (WBCs) and platelets (PLTs), with the rate of deterioration being highly dependent on the anticoagulant. CPDA-1 demonstrated superior preservation, maintaining the most stable WBC and PLT counts. Its metabolic substrates, dextrose and adenine, are crucial for preserving cellular integrity and preventing non-functional platelet activation (Pidcoke *et al*., 2017), thereby retaining critical hemostatic function.

Conversely, Heparin induced the most severe and rapid cellular loss, particularly of platelets, due to cold-induced platelet activation (CIPA) in the absence of metabolic support (Berger *et al*., 2019). This confirms its unsuitability for whole blood preservation. ACD provided intermediate efficacy, superior to Heparin but significantly less effective than CPDA-1.

While leukocyte and platelet degradation is an unavoidable consequence of storage, the use of CPDA-1 significantly mitigates this process, thereby enhancing the quality and therapeutic potential of transfused whole blood.

Table 3: Changes in white blood cell (WBC) and platelet (PLT and PCT) indices over storage time

Storage time (days)	WBC (10^3/mm^3)			PLT (10^3/mm^3)			PCT (mL/L)		
	CPDA-1	ACD	Heparin	CPDA-1	ACD	Heparin	CPDA-1	ACD	Heparin
0	9.57±3.47	10.86^{a} ±2.15	10.5^{a} ±2.30	297.5± 79.1	320.3^{a} ±73.6	320.6^{a} ±62 .0	1.05±0.8 1	1.63±1.37	2.34±2.0 1
1	9.05±2.24	10.89^{a} ±1.94	10.30^{a} ±2.32	287.9± 71.2	317.0^{a} ±75.8	311.7^{a} ±54 .1	1.07± 0.72	1.67±1.37	2.28±2.1 3
3	8.73±1.81	10.85^{a} ±2.06	10.22^{a} ±2.12	293±101.0	301,2 ^{ab} ±66.5	295.2^{ab} ±54.6	1.04±0.8 3	1.64±1.38	2.32±2.1 5
7	8.93±1.95	10.32^{ab} ±1.84	9.27^{ab} ±2.10	259±75.4	288.3 ^{ab} ±62.5	265.5^{b} ±47 .0	1.04± 0.75	1.59±1.35	2.36±2.0 3
14	8.21±1.93	9.83^{b} ±1.91	7.60^{b} ±1.78	265.3± 58.6	257.1^{bc} ± 58.7	214.5^{c} ± 43.2	1.05±0.8 3	1.61±1.40	2.35±2.1 2
21	8.59±3.14	8.59^{b} ±1.66	6.29^{b} ±1.63	249.3± 67.2	224.2^{c} ± 53.9	155.8^{d} ± 35.2	1.10±0.8 1	1.59±1.35	2.32±2.1 4

Note*:* In the same column, values with different superscript letters (ac) indicate statistically significant differences ($p < 0.05$)

4. CONCLUSION

The study confirmed that the choice of anticoagulant has a decisive influence on the quality of dog blood stored for 21 days. Among the three anticoagulants studied, the results showed that CPDA-1 was the optimal preservative, most effectively maintaining the stability of the number, function and morphology of red blood cells, while best limiting the decline of white blood cells and platelets. In contrast, Heparin caused the most severe storage damage, significantly reducing hematological indices after a short time; while ACD showed intermediate effectiveness. Therefore, CPDA-1 should be the first choice in clinical practice at veterinary blood banks to ensure the effectiveness and safety of blood recipient dogs.

Ethical Statement:

In this study, all procedures related to the experimental canine's care, blood testing, were conducted according to the standards and approved by the Animal Ethics Advisory Committee, Hue University, Vietnam (Approval Number: HUVNO49).

Conflict of Interest: The authors declared no potential conflicts of interest relative to the research, authorship, and/or publication of this article.

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