Open Veterinary Journal, (2025), Vol. 15(3): 1253-1263
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Research Article
DOI: *10.5455/OVJ.2025.v15.i3.17*

Submitted: 05/12/2024 Accepted: 22/02/2025 Published: 31/03/2025

A comprehensive overview of fixed-volume hemorrhage effects in New Zealand White rabbit models

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ABSTRACT

Background: Hemorrhagic shock is a life-threatening condition resulting from acute blood loss, leading to compromised tissue perfusion and organ dysfunction. Currently, the guidelines for categorizing and managing hemorrhagic shock in pets are based on protocols developed for humans.

Aim: This study employed New Zealand White rabbits as an animal model to systematically evaluate the physiological and biochemical responses to fixed-volume hemorrhage, aiming to establish its role in inducing shock and significant physiological alterations.

Methods: A total of 21 New Zealand White rabbits, weighing 2–3 kg, were subjected to controlled hemorrhage by withdrawing 30%–35% of their total blood volume via the auricular artery using a 24-G IV catheter over 15 minutes. Parameters were assessed at baseline and 45 minutes post-induction.

Results: Hemorrhage induced significant increases in heart rate and respiratory rate, reflecting compensatory mechanisms to maintain perfusion during shock. The mean arterial pressure and blood pressure significantly declined, consistent with hemorrhagic shock. Oxygen saturation initially decreased but partially recovered over time. All hematological variables decreased. Coagulopathy was indicated by prolonged prothrombin time and activated partial thromboplastin time. Elevated lactate levels indicate a shift to anaerobic metabolism due to hypoxia. The increased levels of interleukin-10 and tumor necrosis factor-alpha suggested an adaptive anti-inflammatory response to mitigate excessive inflammation.

Conclusion: Fixed-volume hemorrhage in New Zealand White rabbits induces the physiological changes characteristic of hemorrhagic shock, providing valuable insights into the pathophysiological response to acute blood loss.

Keywords: Animal model, Coagulation, Fixed volume, Hemorrhage, Inflammation.

Introduction

Hemorrhagic shock is one of the leading causes of death from acute trauma in humans and animals. Rapid and significant blood loss disrupts tissue perfusion, causing vital organs such as the brain, kidneys, and liver to receive insufficient oxygen, leading to organ dysfunction and death (Kislitsina *et al*., 2019; Dutton *et al*., 2023). Although interventions such as fluid resuscitation and blood transfusion are already in use, mortality rates due to hemorrhagic shock remain high (Knight *et al*., 2023).

In veterinary medicine, studies on managing hemorrhagic shock in companion animals like dogs and cats, are still limited, with many approaches adopted from human medical practices. Most data on hemorrhagic shock management in companion animals are derived from human medical literature. However, there are critical differences in the physiological responses between humans and animals, making animal-specific research essential. For instance, the hemodynamic and metabolic mechanisms in dogs and cats may differ from those in humans, meaning that therapies effective for humans may not yield the same results in animals (Tucker *et al*., 2022). Therefore, the use of animal models is crucial for gaining a deeper understanding of the pathophysiology of hemorrhagic shock in animals (Fülöp *et al*., 2013).

Animal models are often used to study naturally occurring diseases in companion animals and to test therapeutic interventions before their wider application

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in veterinary clinics. For example, New Zealand White rabbits have been used in various studies to model hemorrhagic shock due to their physiological responses to bleeding, which are applicable in preclinical research for companion animals (Fowler and Franch, 1957; Schweinburg *et al*., 1959).

Several bleeding models used in animal research include fixed-volume, fixed-pressure, and uncontrolled hemorrhage. This study applied fixed-volume hemorrhage induction to measure the body's compensatory response to blood loss over a specific period. The advantage of this model is its ability to illustrate the hemodynamic response of animals to a particular volume of blood loss, ease of execution, and minimal trauma (Lomas-Niera *et al*., 2005; Moochhala *et al*., 2009). This approach can serve as a basis for establishing standard levels of hemorrhagic shock in animals, particularly companion animals, as an expected outcome of this research. Another benefit of the fixed-volume model is its ability to predict clinical prognosis based on more accurate physiological changes (Frankel *et al*., 2007).

Previous studies by Frankel *et al*. (2007) and Pfeifer *et al*. (2013) used the fixed-volume method but used mice and pigs as animal models, respectively. Mice and pigs are less suitable as models for companion animals, particularly dogs. Mice and dogs have different dopamine responses in renin-angiotensin release as natural vasoconstrictors (Morris *et al*., 2024), whereas pigs and dogs exhibit different hemodynamic responses to synthetic vasoconstrictors. In dogs, these preparations more significantly affect myocardial contractility, whereas in pigs, they influence peripheral resistance (Tune *et al*., 2020).

Other studies by Rezende-Neto *et al*. (2010), Luo *et al*. (2015), Sun *et al*. (2017), and Wang *et al*. (2021) used rabbits but applied fixed-pressure or uncontrolled hemorrhagic shock methods. To date, no study has used rabbits as animal models with fixed-volume hemorrhagic shock induction, nor has any study provided comprehensive data to study physiological responses in companion animals.

Materials and Methods

This study used 21 New Zealand White rabbits weighing 2–3 kg. The treatment began with inhalation anesthesia induction using 3%–4% isoflurane. Hemorrhagic shock was induced by removing 30%–35% of the total blood volume (60 ml x body weight) via the auricular artery using a 24-G intravenous catheter. Blood withdrawal was performed by repeated aspiration until the target volume was reached, and the procedure was completed within 15 minutes. Clinical parameters, including heart rate (HR), respiratory rate (RR), temperature, systolic pressure, diastolic pressure, mean arterial pressure (MAP), oxygen saturation (SPO_2), and end-tidal CO_2 volume ($ETCO_2$), were observed every 15 minutes until the 60 minutes of shock using a patient monitor (Infunix Purescope IP-4050), Blood Pressure Monitor (Contec08A-VET), and Capnograph (Contec® CA10M). Hematology parameters, prothrombin time (PT), activated partial thromboplastin time (aPTT), lactate acid, interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α) were measured by comparing baseline (0 minute) to 60 minutes of shock. Hematology parameters were analyzed using a hematology analyzer (Mindray BC-2800Vet), PT, and aPTT with a QuickVet® Specialty Analyzer™ and QuickVet® PT/aPTT Coag Combo Test™. Lactate levels were measured using the Edge® Lactate Analyzer, and IL-10 and TNF-α levels were assessed using ELISA (Elabscience®). The research data were statistically analyzed using one-way ANOVA followed by Duncan's test with a 95% confidence interval. The data were presented as means and displayed in table form.

Ethical approval

All procedures conducted in this study were approved by the ethics committee of the School of Veterinary Medicine and Biomedical Sciences of IPB University approval number: 033/KEH/SKE/III/2023.

Results

Clinical

The induced bleeding had an effect on variable values at the 0 minute mark post-induction, with a decrease across all variables except RR (Table 1). Some variables demonstrated similar changes from 0 to 60 minutes. The HR variable showed a decrease only at the 0 minute mark, followed by an increase that nearly returned to the baseline value at 0 minute. The temperature and $ETCO_2$ values followed a similar pattern, decreasing but not far from the 0 minute value. Systolic pressure and SpO_2 exhibited fluctuating values, generally trending upward toward the 0 minute level. Diastolic pressure and MAP showed fluctuating changes but tended to decrease, remaining far from the 0 minute value. The RR variable is the only one that demonstrates an increase from 15 to 60 minutes.

Hematology

The results indicate significant changes in various hematological parameters of the animal models experiencing hemorrhagic shock (Table 2), as denoted by different letters (a and b). All hematological variables had decreased values.

Blood Coagulation

The PT variable showed an increase from 18.38 ± 0.14 at minute 0 to 23.33 ± 2.60 at minute 60, indicating a significant prolongation in blood clotting time (Table 3). The differences marked by different letters (a and b) suggest this change is statistically significant. This increase may indicate a disruption of the coagulation process. For the aPTT parameter, although there was an increase from 165.55 ± 12.80 at minute 0 to 223.59 ± 24.55 at minute 60, this change might not be statistically significant, as indicated by the letter "a" (Table 3).

Table 1. Clinical parameters before and after induction of fixed volume hemorrhagic shock in New Zealand White rabbits.

Time (minute)	Variable								
	Heart rate (x per minute)	Respiratory rate (x per minute)	Temp (°C)	Systole (mmHg)	Diastole (mmHg)	Mean arterial pressure (mmHg)	SI	Oxygen saturation (%)	end-tidal CO_2 (mmHg)
0	92.21 ± 5.36^{ab}	56.13 ± 4.49^{a}	39.83 ± 0.08^{a}	110.12 ± 4.90^{a}	56.54 ± 5.94^{b}	81.58 ± 4.24^{b}	0.92 ± 0.11^{a}	94.58 ± 1.19^{d}	14.04 ± 0.77^{a}
15	82.92 ± 5.54^{a}	66.46 ± 6.92^{a}	39.80 ±0.09^{a}	97.58 ± 9.11^{a}	36.92 ± 3.75^{a}	54.13 ± 3.78^{a}	1.14 ± 0.15^{a}	80.33 ± 2.40^{a}	12.29 ± 0.73^{a}
30	87.58 ± 3.77^{ab}	88.00 ± 5.94^{b}	39.76 ± 0.09^{a}	94.83 ± 7.62^{a}	31.25 ± 3.13^{a}	57.54 ± 5.02^{a}	1.09 ± 0.09^{a}	81.58 ± 2.40^{ab}	13.38 ± 0.63^{a}
45	93.13 ± 5.11^{ab}	93.17 ± 5.59^{b}	39.73 ± 0.09^{a}	95.81 ± 6.44^{a}	42.04 ± 5.41^{a}	56.54 ± 5.16^{a}	1.04 ± 0.08^{a}	86.92 ± 2.28^{bc}	12.33 ± 0.71^{a}
60	100.21 ± 5.23^{b}	98.96 ± 5.09^{b}	39.77 ± 0.10^{a}	97.25 ± 7.22^{a}	36.04 ± 4.30^{a}	55.63 ± 4.04^{a}	1.18 ± 0.11^{a}	88.17 ± 2.04^{c}	11.88 ± 0.66^{a}

Data are presented as mean with SD (x ± SE). The same superscript letters in the same row indicate no significant difference ($p > 0.05$)

Table 2. Hematology parameters before and after fixed-volume hemorrhagic shock induction in New Zealand White rabbits.

T (minute)	Variable														
	RBCs (10^6/µl)	Hb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW %	Thrombocyte (10^3/µl)	MPV (fl)	PDW (%)	PCT (%)	WBC (10^3/µl)	Lymphocyte (10^3/µl)	Monocyte (10^3/µl)	Granulocyte (10^3/µl)
0	5.73 ± 0.14a	11.52 ± 0.29a	37.18 ± 0.83a	65.08 ± 0.55	20.05 ± 0.20	30.90 ± 0.20	13.94 ± 0.31	411.05 ± 44.67a	5.60 ± 0.14	16.38 ± 0.12	0.22 ± 0.02a	9.32 ± 2.79a	2.90 ± 0.26	0.38 ± 0.04	4.69 ± 0.96
60	4.33 ± 0.15b	8.53 ± 0.32b	27.96 ± 0.97b	64.67 ± 0.56	19.60 ± 0.19	30.41 ± 0.19	13.65 ± 0.33	251.95 ± 31.86b	5.37 ± 0.12	16.29 ± 0.12	0.15 ± 0.01b	4.60 ± 0.95b	1.60 ± 0.17	0.19 ± 0.03	2.81 ± 0.86

Data are presented as mean SD (x ± SE). The same superscript letters in the same row indicate no significant difference ($p > 0.05$).

Hb = hemoglobin; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; PCT = plateletcrit; PDW = platelet distribution width; RBC = red blood cell; RDW = red cell distribution width; T = time; WBC = white blood cell.

Tabel 3. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and lactate levels before and after fixed-volume hemorrhagic shock induction in New Zealand White rabbits.

Time (minute)	Variable		
	PT (second)	aPTT (second)	Lactic acid level (mg/dl)
0	18.38 ± 0.14[a]	165.55 ± 12.80[a]	27.25 ± 5.05[a]
60	23.33 ± 2.60[b]	223.59 ± 24.55[a]	63.67 ± 6.49[b]

Data are presented as mean with SD (x ± SE). The same superscript letters in the same row indicate no significant difference ($p > 0.05$).

Table 4. Interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-α) levels before and after fixed-volume hemorrhagic shock induction in New Zealand White rabbits.

Time (minute)	Variable	
	IL-10 (pg/ml)	TNF-α (pg/ml)
0	27.10 ± 0.89[a]	27.00 ± 3.87[a]
60	30.96 ± 1.39[b]	28.35 ± 4.12[a]

Data are presented as mean with SD (x ± SE). The same superscript letters in the same row indicate no significant difference ($p > 0.05$).

Inflammatory mediators

Table 4 presents the levels of IL-10 and TNF-α in New Zealand White rabbits before (0 minute) and after fixed-volume hemorrhagic shock induction (60 minutes). The IL-10 level increased significantly from 27.10 ± 0.89 pg/ml at 0 minute to 30.96 ± 1.39 pg/ml at 60 minutes, indicating a stronger anti-inflammatory response following hemorrhagic shock induction. In contrast, the TNF-α level did not differ significantly between 0 minute (27.00 ± 3.87 pg/ml) and 60 minutes (28.35 ± 4.12 pg/ml), indicating that hemorrhagic shock did not significantly affect TNF-α release during this period. These findings suggest that in this hemorrhagic shock model, the anti-inflammatory response mediated by IL-10 is more dominant than the pro-inflammatory response triggered by TNF-α within the first 60 minutes after induction.

Discussion

The changes induced by hemorrhagic shock (30%–35%) in this study are similar to those observed in previous research (Rahmiati *et al*., 2023). Despite differences in the blood withdrawal site and volume extracted, the clinical effects were comparable. An increase in HR after shock induction occurs due to baroreflex receptor activation, which triggers the renin-angiotensin system and adrenaline release. This physiological response aims to increase the HR and ensure tissues and cells receive an adequate blood supply. Additionally, this response helps maintain blood pressure and optimizes vital organ perfusion, which is critical in emergencies like hemorrhagic shock (Schultz and McConachie, 2015).

The increase in the RR involves more specific cellular mechanisms. Blood loss leads to an unmet oxygen demand in tissues, causing mitochondria to shift from aerobic to less efficient anaerobic metabolism for adenosine triphosphate (ATP) production. During this process, pyruvate is produced and converted to lactic acid to regenerate nicotinamide adenine dinucleotide (NAD+), thereby maintaining partial cellular respiration despite oxygen scarcity. This shift to anaerobic metabolism results in lactic acid accumulation, potentially causing blood pH to drop (acidosis). To compensate, the body increases its respiration rate to expel carbon dioxide (CO_2) and reduce blood acidity. This hyperventilation response aims to create respiratory alkalosis by removing CO_2 from the blood (Convertino *et al*., 2019). This compensatory mechanism helps maintain acid-base balance and ensures tissue oxygenation despite hemorrhagic shock (Hooper and Armstrong, 2022).

Temperature changes showed a declining trend but remained close to the baseline. The gradual decrease in temperature results from the bleeding process itself. In the acute phase, blood flow to the skin and mucosal surfaces diminishes, potentially reducing metabolism and increasing heat loss, potentially leading to hypothermia. Body temperature regulation involves balancing heat production and loss. In mammals, heat is a byproduct of metabolism. The mechanisms for maintaining this balance include convection, conduction, evaporation, and radiation (Henderson *et al*., 2000). In rabbits, heat loss through radiation and convection primarily occurs via the ears (Yuan *et al*., 2022). In other species, heat loss pathways may vary (Jordan, 1995).

Systolic pressure changes tended to fluctuate, with a decrease followed by an increase toward baseline at minute 0. Systolic pressure is influenced by HR.

A decrease in HR typically lowers systolic pressure (Priestley *et al*., 2019). Baroreflex receptor activation-induced increase in HR raises systolic pressure. Unlike systolic pressure, diastolic pressure responded differently, showing fluctuating declines until minute 60, which is far from the baseline value. This could result from the acute hemorrhagic shock response, involving positive modulation of chronotropy (sinoatrial node conductance), dromotropic (atrioventricular node conductance), and negative lusitropic (relaxation), marked by HR elevation (Ranjan and Gulati, 2023). During acute hemorrhagic shock, the priority is to pump blood rapidly to tissues, quickly filling myocardial vasculature during systole. Conversely, during diastole, slower ventricular vascular filling increases the risk of diastolic dysfunction. Several studies have found that hemorrhagic conditions can impair myocardial contractility and cause diastolic dysfunction (Suzuki *et al*., 1995). Elansary *et al*. (2022) demonstrated that the severity of hemorrhagic shock is correlated with greater left ventricular dysfunction and reduced myocardial blood flow. According to D'Annunzio *et al*. (2012), diastolic pressure below 40 mm Hg indicates subendocardial ischemia.

The MAP changes mirrored those of diastolic pressure. MAP is influenced by cardiac output and systemic vascular resistance. Blood volume loss reduces vascular lumen pressure and cardiac output. Physiologically, rapid blood loss lowers MAP during the first 10 minutes and gradually increases afterward, provided no recurrent bleeding occurs (Frankel *et al*., 2007). In this study, the body seemed unable to compensate for blood loss by increasing MAP, possibly due to the physiological limits of MAP elevation after hemorrhage. Scully *et al*. (2016) found that MAP elevation after hemorrhage occurs within the first 15 minutes after bleeding, with a modest increase of around 20 mm Hg compared with the initial 30 mm Hg decrease.

Changes in HR, systolic pressure, diastolic pressure, and MAP can predict cardiac function. However, these variables are best evaluated in combination. For instance, HR was associated with systolic pressure, MAP, or diastolic pressure. Acute arterial pressure drops are usually counteracted by sympathetic nervous system activity, although compensation can sometimes become maladaptive. Therefore, ratios such as HR to systolic pressure (shock index, SI) or HR to MAP can help identify underlying dysfunctions. SI is a good predictor of mortality, although it has low sensitivity in geriatric and obstetric patients (Kamikawa and Hayashi, 2020). A higher SI indicates more severe shock.

The SI in this study was 1.18 ± 0.11. In humans, SI-based shock classifications are as follows: Class I (no shock): SI < 0.6; Class II (mild shock) : SI ≥ 0.6 to < 1.0; Class III (moderate shock) : SI ≥ 1.0 to < 1.4; and Class IV (severe shock) : SI ≥ 1.4 (Mutschler *et al*., 2013). However, these values may not be directly applicable to animals. Further evaluation of clinical parameters is necessary to establish shock classification in animals.

Unlike the SI, the HR-to-diastolic pressure ratio (diastolic index, DAI) predicts circulatory dysfunction during vasodilation (Ospina-Tascón *et al*., 2020). DAI values were not analyzed in this study. Based on these results, the HR increase did not compensate for the diastolic pressure drop, likely due to a gradual reduction in vascular tone (Ospina-Tascón *et al*., 2020; Han *et al*., 2022). DAI is commonly used to predict sepsis severity or post-resuscitation conditions (Han *et al*., 2022). As previously mentioned, a drop in diastolic pressure indicates myocardial contractility dysfunction, which requires confirmation through ejection fraction assessment via ultrasound (Carrara *et al*., 2018). This was not performed in the present study, representing a limitation.

Comparatively, myocardial contractility dysfunction usually appears 2 to 5 hours post-shock (Meng *et al*., 2005; Carrara *et al*., 2018). Compensation during shock without subsequent myocardial dysfunction is typically observed with hemodynamic changes, such as systolic and MAP decreases, while diastolic pressure is maintained. This finding differs from that of this study, suggesting that shock induction likely causes myocardial contractility dysfunction, which is potentially reversible.

SPO_2 changes mirrored systolic pressure patterns, with end-study values < 90%. In humans, SPO_2 < 90% indicates poor prognosis and severe shock (Qi *et al*., 2020). SPO_2 reductions result from decreased blood volume and hemoglobin (Hb), lowering blood oxygen levels. SPO_2 reflects microcirculation, tissue perfusion, and oxygenation (Raux *et al*., 2006). Factors like acidic pH, increased CO_2, and red blood cell (RBC) glycolysis affect Hb's oxygen affinity post-shock (Fecher *et al*., 2021).

$ETCO_2$ reflects exhaled CO_2 levels at the end of expiration, indicating the sufficiency of CO_2 transport in the blood. In this study, $ETCO_2$ changes followed temperature patterns, with slight decreases from baseline. This variable is closely linked to RR. The study noted that RR increases with hyperventilation to create respiratory alkalosis by expelling CO_2, neutralizing H^+ accumulation, and counteracting pH decreases. Respiratory alkalosis is reflected by simultaneous $ETCO_2$ decreases (Convertino *et al*., 2019).

The clinical changes observed in this study are consistent with the patterns reported in hemorrhagic shock induction via carotid or femoral artery catheterization (Luo *et al*., 2015; Wang *et al*., 2021; Sun *et al*., 2017;). This demonstrates that the New Zealand White rabbit model, induced using a fixed-volume and minimally invasive method in this study, can exhibit clinical changes similar to those observed with the fixed-pressure method, which typically results in greater trauma.

The induction of hemorrhage through blood withdrawal resulted in the loss of blood components, including RBCs, platelets, and WBCs (white blood cells) (Constable *et al*., 2016). A decrease in Hb occurred immediately as the RBC count decreased, and a similar decrease in Hct was observed as blood components were reduced. In this study, the reduction in all hematological variables ranged from 22% to 50%. This downward trend was observed for almost all variables except for the percentage of lymphocytes and granulocytes. The normal hematological ranges for NZW rabbits, the RBC, Hb, packed cell volume (PCV), mean corpuscular hemoglobin concentration, platelet, and WBC values at 60 minutes were below the normal range (Moore *et al*., 2021). This condition indicates anemia, thrombocytopenia, and leukopenia, collectively known as pancytopenia.

Anemia caused by trauma or hemorrhage can persist for several weeks to months. This condition resembles chronic inflammatory anemia and is characterized by low reticulocyte counts, high erythropoietin levels, and dysfunctional iron regulation (Kelly *et al*., 2021). Furthermore, anemia may persist longer due to changes in RBC size and shape resulting from shock-induced oxidative stress (Berezina *et al*., 2001). Factors such as oxygen free radicals, toxins, ATP depletion, changes in intracellular ionic composition, and complement activation reduce RBC deformability and alter their morphology (Zaets *et al*., 2003). Variables like mean corpuscular volume and red cell distribution width are used to analyze RBC size and shape changes, but in this study, they showed minimal variation.

Studies on the morphological changes in RBCs in response to hemorrhage vary widely. According to Schumacher (1992), RBC shape changes occur 1–2 weeks after hemorrhage, whereas Berezina *et al*. (2001) observed such changes within minutes. The extent of RBC morphological changes depends on the severity and duration of hypotension (Berezina *et al*., 2001). Hb levels follow a similar trend as RBC counts, with decreases observable within minutes. In humans, Hb levels below 10 g/dl within 30 minutes post-hemorrhage can indicate the presence of significant bleeding sources (Bruns *et al*., 2007; Figueiredo *et al*., 2018).

Thrombocytopenia following trauma or hemorrhage is associated with consumptive processes and dilutional effects. After injury, platelets are extensively utilized for clot formation to halt bleeding, thereby reducing their availability in circulation. Dilutional effects can occur even before resuscitation because hypotension induces fluid reabsorption to maintain blood flow. This reabsorption leads to the dilution of cellular components in the microvasculature, a process known as autotransfusion (Woodcock and Woodcock, 2012; Michel *et al*., 2020; Munoz *et al*., 2020).

WBC differential counts all decreased compared with baseline values. This result differs from those of Gaylor *et al*. (1969) and Hawksworth *et al*. (2012), who reported increased neutrophil and lymphocyte counts post-hemorrhage. The discrepancy may arise from differences in blood collection timing post-hemorrhage and the physical factors affecting the vascular lumen. Neutrophil mobilization from marginal to circulating pools occurs within minutes to hours (Tvedten and Raskin, 2011). However, according to Suwa *et al*. (2000), biphasic neutrophilia occurs at approximately 3 and 9 hours post-induction of inflammatory mediators. In this study, blood was collected 1 hour after hemorrhage, likely before peak neutrophil levels were reached. Normally, lymphocyte counts increase in response to hypoxia (Kokura *et al*., 2000) under the influence of inflammatory mediators, including those induced by post-hemorrhagic hypoxia. Moreover, monocyte mobilization is triggered by inflammatory mediators (Qin *et al*., 2020). These responses occur within minutes to hours and are correlated with increased vascular permeability that allows neutrophils, lymphocytes, and monocytes to circulate (Jian *et al*., 2019; Qin *et al*., 2020). However, in this study, these responses were likely not observed within the limited observation period.

Lactate levels also showed a significant increase from 27.25 ± 5.05 at minute 0 to 63.67 ± 6.49 at minute 60. This significant difference, indicated by different letters (a and b) (Table 3), suggests an increase in lactate production, possibly due to elevated anaerobic metabolic activity or hypoxic conditions. These results indicate a significant metabolic disturbance alongside coagulation process disruption, as seen in the increase in PT. Overall, these changes demonstrate a significant physiological response over time.

PT and aPTT are tests used to detect abnormalities in blood coagulation, each evaluating different pathways: PT assesses the extrinsic pathway, while aPTT evaluates the intrinsic pathway (Ford and Maazafeero, 2012). In this study, an increase in PT values or prolonged clotting time was observed. Generally, coagulation abnormalities are indicated when the PT or aPTT values exceed 1.5 times the upper limit of the reference interval (Condrey *et al*., 2020). In this study, the 0 minute value was used as the reference. The increase observed 45 minutes after induction was 1.3 times the 0 minute value. Although this does not indicate a coagulation disorder, it does show a statistically significant difference compared with the 0 minute PT value. Prolonged PT is associated with the occurrence of hypocoagulability (Moore *et al*., 2021; Tuan *et al*., 2021).

During trauma or bleeding, exposed tissue factors initiate thrombin and clot formation. Platelets are activated through cellular signaling involving collagen, von Willebrand factor (vWF), and glycoproteins, which enhance coagulation and deplete fibrinogen, and factor V. Fibrinolysis also occurs when plasminogen is converted into plasmin. This leads to hypocoagulation

and hyperfibrinolysis in post-trauma or hemorrhagic patients (Loscalzo & Schafer, 2003; Shaz *et al*., 2011). In this study, aPTT values also showed an increase or prolonged clotting time. Similar to the PT values, the aPTT increased by approximately 1.3 times from 0 minute, although it was not significantly different from the 0 minute mark. The slight prolongation in PT and aPTT suggests a moderate decrease in procoagulant (Moore *et al*., 2021). Physiologically, a decline in procoagulant may accompany increased thrombin, marking clot formation at required injury sites. Excessive thrombin formation plays a crucial role in delayed hypercoagulability in patients with trauma or hemorrhage. In severe trauma and unresolved bleeding, circulating thrombin accumulates, shifting the state to hypercoagulability, which is termed trauma-induced coagulopathy (TIC) (Dunbar and Chandler, 2009; Woolley *et al*., 2020).

Lactate levels are commonly monitored to assess tissue perfusion status. Lactate is produced by most tissues, and its highest production occurs in the muscles. Under normal conditions, lactate is quickly metabolized by the liver and, to a lesser extent, the kidneys. Aerobically, pyruvate is produced via glycolysis and enters the Krebs cycle, whereas anaerobically, lactate is the final glycolysis product, serving as a substrate for gluconeogenesis (Andersen *et al*., 2013).

In this study, lactate levels increased 2.3 times at 45 minutes post-induction compared with the 0 minute mark, showing a statistically significant increase. Hemorrhagic shock leads to reduced blood volume, affecting the cellular level. Microcirculation suffers from reduced tissue perfusion, subsequently inducing anaerobic metabolism and acid production, causing acidosis (Munoz *et al*., 2020). Acidosis, in turn, impacts blood coagulation (Engström *et al*., 2006). Acidosis causes plasma protein dysfunction and rapid fibrinogen degradation, disrupting nearly all coagulation stages under this condition (Savioli *et al*., 2021). In humans, pH below 7.4 can lead to platelet shape and structural changes, reduced factor activity, and impaired thrombin production (Martini *et al*., 2005). It also decreases fibrinogen concentration, increases fibrinogen degradation (due to fibrinolysis and elevated factor XIII), and does not affect fibrinogen production while promoting a pro-inflammatory response mediated by platelets (Etulain *et al*., 2012). Bicarbonate administration to correct acidosis is not correlated with TIC reversal (Martini *et al*., 2006). Animal studies and in vitro experiments have shown that acidosis inhibits fibrin polymerization, decreases factor V and IX activity, reduces platelet aggregation, increases fibrinogen consumption, lowers platelet count, reduces thrombin formation, weakens clot strength, and produces abnormal conventional coagulation test results (Martini *et al*., 2005; Martini and Holcomb, 2007). As hemorrhagic shock progresses, hypercoagulability occurs due to prothrombotic changes and the cessation of fibrinolysis, which increases organ damage by producing thrombi and clogging microvascular circulation, ultimately leading to organ failure (Moore *et al*., 2021).

Both IL-10 and TNF-α showed an increase compared to the 0 min mark (Table 4). This change is consistent with Bahrami *et al*. (1997) and Schneider *et al*. (2004). In the post-hemorrhagic shock mechanism, TNF-α and IL-10 play crucial roles in regulating the inflammatory response. TNF-α is one of the first pro-inflammatory cytokines released after injury, initiating the early inflammatory response and stimulating the release of other cytokines like IL-1 and IL-6 (Bahrami *et al*., 2017). Although essential for initiating tissue repair, excessive TNF-α elevation can lead to further tissue damage and increase the risk of multiple organ failure (Kirchhoff *et al*., 2009; Villarroel *et al*., 2023).

Conversely, IL-10 functions as an anti-inflammatory cytokine, controlling and suppressing the inflammatory response by inhibiting the production of TNF-α and IL-6 (Schneider *et al*., 2004). In the early phase following hemorrhagic shock, IL-10 acts to balance the pro-inflammatory effects triggered by TNF-α to prevent uncontrolled inflammation (Schwacha *et al*., 2001). However, excessive release of IL-10 can also have adverse effects because it may cause immunosuppression, increasing the body's susceptibility to secondary infections (Ayala *et al*., 1994). The balance between TNF-α and IL-10 is critical in determining post-hemorrhagic shock outcomes, as an excessively high or low inflammatory response can lead to serious complications, such as organ failure or infection (Giannoudis *et al*., 2000).

Conclusion

Research on fixed-volume hemorrhage in New Zealand White rabbits demonstrates significant physiological responses characteristic of hemorrhagic shock, providing valuable insights into the body's pathological adaptation to acute blood loss. This model demonstrates how blood pressure, HR, oxygenation, and tissue perfusion are affected during controlled hemorrhage, simulating a shock state similar to human responses. These findings highlight the usefulness of New Zealand White rabbits as a translational model for hemorrhagic shock research in companion animals. The results of this study can be used to establish more detailed shock level standards for companion animals in accordance with clinical criteria and blood profiles.

Acknowledgments

The authors would like to express their sincere gratitude to the Veterinary Teaching Hospital, including the Division of Surgery and Radiology and the Division of Pathology, School of Veterinary Medicine and Biomedical Sciences, IPB University, for providing the research facilities and location for this study.

Appreciation is also extended to PT Zoetis Indonesia for their valuable technical support.

Conflict of interest

The authors declare no conflict of interest.

Funding

The authors gratefully acknowledge the financial assistance provided by the Center for Higher Education Funding (BPPT) and the Indonesia Endowment Fund for Education (LPDP), as stated in decree number 01802/J5.2.3/BPI.06/9/2022.

Authors' contributions

DUR: general study design, data collection, data interpretation, manuscript drafting. G: design of shock induction in animal models. DN: interpretation of clinical data. RHS: reviewing manuscript drafting. EH: interpretation of pathological immune response.

Data availability

All related data is presented in the text.

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