

REPRESENTATION OF ERYTHROCYTES IN PIGLET (*Sus scrofa*) POST HYPOVOLEMIC SHOCK WITH NORMO AND HYPERVOLEMIA RESUSCITATION OF CRYSTALLOID FLUID

Gunanti Soedjono^{1,2*}, Ahmad Arif Amin³, Antonius H. Pudjiadi⁴, Melpa Susanti Purba¹, and Najma Hayati¹

¹Department of Veterinary Clinic Reproduction and Pathology, Division of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia

²Veterinary Paramedic Study Program, Directorate of Diploma Programs, Bogor Agricultural University, Bogor, Indonesia

³Department of Parasitology and Medical Entomology, Department of Animal Diseases and Veterinary Health, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia

⁴Department of Pediatric, Faculty of Medicine, University of Indonesia, Depok, Jawa Barat, Indonesia

*Corresponding author: gunanti.soe@gmail.com

ABSTRACT

The aim of this study is to evaluate the representation of erythrocytes, which is the count of erythrocytes; hemoglobin concentration, hematocrit value, and platelet count in piglet (*Sus scrofa*) after hypovolemic shock with normal and hypervolemia resuscitation of crystalloid fluid. This study uses nine male castrated piglets 6-8 weeks of age. The piglets are given treatments such catheter installation, shock induction, normovolemia resuscitation, and hypervolemia resuscitation. The fluid for resuscitation is crystalloid fluid, NaCl 0.9%. Blood sample is taken in baseline, normovolemia condition, and hypervolemia condition. The sample is analyzed using hematology blood analyzer and is tested using T-Paired. Total erythrocytes count after normovolemia resuscitation is $3.07 \times 10^6/\mu\text{L}$, while after hypervolemia resuscitation the erythrocytes count decrease until $2.86 \times 10^6/\mu\text{L}$. Hemoglobin concentration after normovolemia resuscitation is 9.4 g/dL, while after hypervolemia resuscitation hemoglobin concentration decreases to 8.64 g/dL. Hematocrit count from piglets after normovolemia resuscitation is 28%, while it decreases after hypervolemia resuscitation to 25.89%. Platelet count after normovolemia resuscitation is $297.22 \times 10^3/\text{mm}^3$ and decreases to $263.89 \times 10^3/\text{mm}^3$ after hypervolemia resuscitation. Evaluation of the erythrocytes index shows that the piglets suffer anemia. Hypovolemic shock and normal, and hypervolemia resuscitation cause the significant decreases in all erythrocytes indexes except the platelet count.

Key words: crystalloid, erythrocytes, fluid resuscitation, hypovolemia, shock

ABSTRAK

Tujuan dari penelitian ini adalah mengevaluasi gambaran variabel sel darah merah meliputi jumlah sel darah merah, konsentrasi hemoglobin, nilai hematokrit, dan nilai platelet pada anak babi (*Sus scrofa*) pasca keadaan syok hipovolemik dengan resusitasi cairan kristaloid normo dan hipervolemia. Penelitian ini menggunakan sembilan anak babi jantan kastrasi berusia 6–8 minggu. Perlakuan pada babi meliputi pemasangan kateter, induksi syok, resusitasi normovolemik, dan resusitasi hipervolemia. Resusitasi cairan menggunakan cairan kristaloid NaCl 0.9%. Pengambilan sampel darah dilakukan pada saat baseline, pascaresusitasi normovolemik, dan pascaresusitasi hipervolemia. Sampel darah dianalisis menggunakan hematology blood analyzer dan diuji menggunakan T-Paired Sample Test. Total jumlah eritrosit setelah resusitasi normovolemik adalah $3,07 \times 10^6/\mu\text{L}$, sedangkan setelah resusitasi hipervolemia, jumlah eritrosit menurun hingga $2,86 \times 10^6/\mu\text{L}$. Konsentrasi hemoglobin setelah resusitasi normovolemik adalah 9,4 g/dl, sedangkan setelah resusitasi hipervolemia konsentrasi hemoglobin menurun menjadi 8,64 g/dl. Jumlah hematokrit dari anak babi setelah resusitasi normovolemik adalah 28%, sementara itu berkurang setelah resusitasi hipervolemia menjadi 25,89%. Jumlah trombosit setelah resusitasi normovolemik adalah $297,22 \times 10^3/\text{mm}^3$ dan menurun menjadi $263,89 \times 10^3/\text{mm}^3$ setelah resusitasi hipervolemik. Hasil evaluasi gambaran sel darah merah anak babi menunjukkan terjadinya anemia pada seluruh babi. Kondisi syok hipovolemik dan resusitasi normo dan hipervolemia menyebabkan penurunan yang signifikan pada seluruh variabel sel darah merah kecuali nilai platelet.

Kata kunci: kristaloid, sel darah merah, resusitasi cairan, hipovolemik, syok

INTRODUCTION

Pigs are the main model animals for humans due to their similarities in size and physiology, and the similarities in organ development and disease progression (Lunney, 2007; Bassols *et al.*, 2014). Shock is the clinical expression of circulatory failure. This condition is a result of inadequate cellular oxygen. Shock can result from several mechanism: hypovolemia, cardiogenic factors (acute myocardial infarct and end-stage cardiomyopathy), obstruction (embolism and cardiac tamponed), or distributive factors (sepsis and anaphylaxis) (Vincent and Backer, 2013). Based on this, the shock can be grouped into the cardiogenic shock, the hypovolemic shock, and the distributive shock (sepsis) (Standl *et al.*, 2018).

Hypovolemic shock can occur because of uncontrolled blood loss after severe injury.

Hemorrhagic shock can cause hypotension in all trauma patients. Shock often leads to mortality and have a major risk of complications and multiple organ dysfunctions (Kudo *et al.*, 2017). Patients experience hypovolemic shock can be identified by elevated heart rate, low blood pressure, narrowed pulse pressure, and decreased capillary refill, cool clammy extremities, pale skin, increased skin turgor, dry mucus membrane, and low urine output (Kobayashi *et al.*, 2012). The trauma injuries are the main cause of the death of patients aged one to forty years in the United States. The United States military estimates that 90,000 people died in the United States due to the traumatic injuries and 66-80% of these deaths resulted from bleeding (Spinella and Holcomb, 2009).

Hematology test is important to determine hematologic disorder, health status, and often being the most important diagnostic information. The major

function of erythrocytes is to carry the oxygen and distribute it to whole body (Greer *et al.*, 2009). The incidence of large amounts of fluid loss caused by bleeding is feared to affect the variable blood balance in the patients. For this reason, it is necessary to do research on the description of red blood cells in animals that experience the hypovolemic shock.

MATERIALS AND METHODS

This research had received the ethical approval from the Animal Ethics Commission of the Faculty of Veterinary Medicine, Bogor Agricultural University with the SKHE No. 055/KEH/SKE/III/2017. The materials used are 9 piglets (*Sus scrofa*) aged 6-10 weeks with the male gender castration and the body weight 10-20 kg. The pigs are in a healthy clinical condition, fasted overnight before the surgery and are still given *ad libitum*.

Animal Acclimatization

The acclimatization is carried out in the Animal Cage, Laboratory Animal Management Unit of the Faculty of Veterinary Medicine, Bogor Agricultural University. During the acclimatization process, the animals are fed twice a day and *ad libitum* drinking water. The animals are given the oxfendazole (Vermo O[®], Oxfendazole Sanbe) worm medication given orally by mixing it with the pig feed and for the animals that show the symptoms of illness such as coughing, the oxytetracycline 20% (Vet-Oxy La[®], Sanbe) antibiotic are administered at a dose of 10 mg/kg bw parenterally by i.m. route. The animal cages are cleaned in the morning and the evening. The acclimatization is carried out with the aim that the animals adapt to the new environment during the research.

Handling of PiCCO Tools

The preparation phase for the surgery begins with the anesthesia of the animals in the cage using 20 mg/kg ketamine 10% (Ket-A-100[®], Agrovat) and 2 mg/kg xylazine 10% (Ilium Xylazine-100[®], Ilium) in i.m. in the femoral area. Then after the animals fall asleep, the animals are taken to the experimental surgery room to be weighed and measured in their body lengths. These weighing are done to facilitate the calculation of the anesthetic doses. Then the preparation is done, which is cleaning all the body parts of the animal, especially shaving the hair on the ears, the neck, the ventral, the tail, and the medial back legs. After that, the animal is cleaned with detergent and water and then given an antiseptic in the form of iodine (Povidone Iodine[®], One Med). This cleaning or preparation is done to facilitate the installation of i.v. catheter and the installation of patient monitoring and Pulse Contour Cardiac Output (PiCCO).

Intra vena catheter 22G and three-way stop cock are mounted on the auricularis vein and then the animal is transferred to the operating table, carried out with a rope and endotracheal tube (ETT) which is installed with the help of laryngoscope. Previously, pigs are

given the topical lidocaine (Lidocaine 2%, Phapros) to the epiglottis and then ETT is connected with the oxygen hose and ventilator. The saturation measuring device is installed on the tail that has been prepared in advance and the infusion of 0.9% i.v. NaCl (Ecosol[®], B Braun) through the auricularis vein is carried out. Next is installed the PiCCO tool. Searching for PiCCO access is done through small surgery in the ventral area of the neck and the medial thigh to look for the internal jugular vein and the femoral artery. Immediately after the access is obtained, the PiCCO catheter (Pulsioath, Pulsion Medical System AG) is installed and fixed with the silk thread. After being installed, a tool test is conducted by setting the variables and then measuring the CO curve with the bolus NaCl 0.9% at the temperature of <8° C.

Making Hypovolemic Shock

The shock conditions are characterized by a decrease in Mean Arterial Pressure (MAP) of 20% of the initial value based on the fixed-pressure hemorrhage method. The decrease in MAP is done by removing the blood through the internal jugular vein using a 50 mL syringe.

Resuscitation of the Hypervolemia Crystalloid Fluid

Resuscitation is the phase after the hypervolemia shock. The resuscitation is done by administering the crystalloid fluid of 0.9% NaCl i.v. through the internal jugular vein, the amount of blood released at the phase of making the shock. NaCl intake is done slowly using a 50 mL syringe. The hypervolemia phase is the next phase, and it is done by entering the crystalloid fluid of 0.9% NaCl i.v., the amount of 40 mL/kg bw using 50 mL syringe through the internal jugular vein.

Data Retrieval and Processing

Data collection is carried out by taking the blood sample from the internal jugular vein of the pigs during the baseline condition (initial before treatment), the normovolemia (after a number of shock volume resuscitation), and the hypervolemia (after the hypervolemia resuscitation). The blood samples are stored in the vacutainer containing the ethylenediaminetetraacetic acid (EDTA) anticoagulants. The use of EDTA anticoagulants is chosen because EDTA is able to maintain the cellular components and the blood morphology. The variable blood analysis is performed using the hematology blood analyzer in the laboratory.

Data Analysis

The data measurement results are expressed in the means and the standard deviations. The data are tested with T-paired sample test with the confidence interval of 95%.

RESULTS AND DISCUSSION

Number of Red Blood Cells

The results of the calculation of the number of red blood cells obtained in this study indicate a significant

difference between the time of sampling (Table 1). The mean number of the piglet red blood cells at the baseline, normovolemia, and hypervolemia conditions are below the normal range. The normal value of the red blood cell count in the piglets is $5\text{--}7 \times 10^6/\mu\text{L}$ (Semiadi and Nugraha, 2009). This shows that the piglets have experienced the anemic condition from the beginning.

Hemoglobin Level

Hemoglobin consists of four iron atoms (Fe) and a protein called heme. The function of hemoglobin is to bind the oxygen during the transport process by the red blood cells. The normal hemoglobin level in the piglets is 9-13 g/dL (Plowman and Smith, 2008). The observations obtained show that there are significant differences between the mean hemoglobin level at the baseline, normovolemia, and hypervolemia conditions (Table 2).

The hemoglobin level in the baseline and normovolemia conditions is in the normal range, but in the hypervolemia condition the hemoglobin falls below the normal range. The decrease in the hemoglobin value under the hypervolemia condition can occur due to the use of the crystalloid fluid during the resuscitation. According to Gutierrez *et al.* (2004), the aggressive fluid replacement can result in an isovolemic anemia condition that is adequate blood volume but low hemoglobin concentration and oxygen transport capacity. The condition of isovolemic anemia or relative anemia results from the blood dilution after the resuscitation using the crystalloid fluid. The piglets

are the animals susceptible to iron (Fe) deficiency conditions. The low levels of iron in the body and the low iron content in the mother's milk result in a lack of iron intake in the piglets. Other than that, the limited access of pigs with the land which can be an additional source of iron, has caused this deficiency condition to worsen. This state of iron deficiency is a major cause of the anemia in piglets (Perri *et al.*, 2016).

Hematocrit Value

The results of the mean hematocrit value show a significant difference between the baseline, normovolemia, and hypervolemia conditions (Table 3). The range of normal hematocrit values of piglets is 36-43% (Semiadi and Nugraha, 2009). Based on the results of observation in Tables 1 and 3, the piglets used in this study are suspected of having the anemia from the start. Anemia is an insufficiency condition of the erythrocytes to deliver oxygen to the cells (Greer *et al.*, 2009). The anemia is divided into four, namely the anemia due to bleeding, the impaired erythrocyte production, the increased erythrocyte destruction, and the nutritional deficiency.

The mean of hematocrit value in the baseline condition is already under the normal range of $30.11 \pm 2.03\%$ of the minimum value of 36%. This indicates the occurrence of anemia in the piglets from the beginning before the treatment, as illustrated by the value of the number of the red blood cells (data in Table 1). As stated by Rhoades and Bell (2009), the decrease in hematocrit values indicates the incidence of anemia and often shows the occurrence of the blood

Table 1. The mean number of the red blood cells ($\times 10^6/\mu\text{L}$) before and after the normo and hypervolemia crystalloid fluid resuscitation in the experimental animals

Time of sampling	Number of red blood cell
Baseline	3.32 ± 0.18^a
Normovolemia	3.07 ± 0.27^b
Hypervolemia	2.86 ± 0.23^c

^{a, b, c}Different superscripts within the same column indicate significant differences ($P < 0.05$)

Table 2. The mean of hemoglobin level (g/dL) before and after the resuscitation of the normo and hypervolemia crystalloid fluid in the experimental animal

Time of sampling	Hemoglobin level
Baseline	10.16 ± 0.76^a
Normovolemia	9.40 ± 0.82^b
Hypervolemia	8.64 ± 0.61^c

^{a, b, c}Different superscripts within the same column indicate significant differences ($P < 0.05$)

Table 3. The mean of hematocrit (PCV) (%) value before and after the normo and hypervolemia crystalloid fluid resuscitation in the experimental animals

Time of sampling	Hematocrit value
Baseline	30.11 ± 2.03^a
Normovolemia	28.00 ± 2.29^b
Hipervolemic	25.89 ± 1.97^c

^{a, b, c}Different superscripts within the same column indicate significant differences ($P < 0.05$)

Table 4. The mean of platelet number ($\times 10^3/\text{mm}^3$) before and after the normo and hypervolemia crystalloid fluid resuscitation in the experimental animals

Time of sampling	Number of platelet
Baseline	$263.22 \pm 112.11^{a,b}$
Normovolemia	297.22 ± 109.12^a
Hypervolemia	263.89 ± 90.23^b

^{a, b}Different superscripts within the same column indicate significant differences ($P < 0.05$)

loss as a result of bleeding or deficiency in the production of the red blood cells.

In the shock condition, there is a noticeable decrease in the mean of the number of the erythrocytes, hemoglobin, and hematocrit. The decrease in the number of erythrocytes can occur due to the bleeding, which is a large amount of the blood volume lost from the body. The hemoglobin level and the hematocrit value decrease due to the resuscitation action using the crystalloid fluid which is intended to replace the lost blood volume (Gutierrez *et al.*, 2004).

Continuous reduction in the hemoglobin level can be fatal, because the hemoglobin is a functional unit in the red blood cell that plays a role in transporting the oxygen to tissues (Yoshida *et al.*, 2019). This decrease in the hemoglobin level will cause the amount of oxygen circulating to decrease. The consequence of this decrease in the amount of oxygen which is circulating is the occurrence of hypoxia in the tissues.

Number of Platelet

According to Rumbaut and Thiagarajan (2010), the platelet is part of the blood cell that plays a role in the blood clotting in the area of injury in the blood vessels. In the normal circulation, the platelet is at rest and will be activated when the vascular damage occurs (Berndt, 2000). The results of observing the platelet number in all three conditions show a marked difference between the normovolemia and hypervolemia conditions, but the baseline condition is not significantly different from the normovolemia and hypervolemia conditions (Table 4).

The normal range of platelet in the piglets is $200-500 \times 10^3/\text{mm}^3$ (Semiadi and Nugraha, 2009). The platelet numbers in all three conditions are in the normal range, so that it can be said that the animals do not experience thrombocytopenia and thrombosis after the treatment. Normally, the platelet numbers show that the treatment of hypervolemia fluid resuscitation does not cause damage to the vascular endothelium.

The normovolemia condition is a condition in the animals that have been resuscitated using 0.9% NaCl in the amount of blood volume lost during the shock. In this condition, the number of animal platelet increases when other variables decrease in value. However, this increase in the platelet value is still within the normal range and not significantly different. The increase in the platelet number indicates the damage to the arteries, according to the statement of Berndt (2000), namely in the normal circulation, the platelet is at rest and will be activated when the vascular damage occurs.

According to Pascual *et al.* (2002), the resuscitation using the isotonic crystalloid fluid can increase the blood vessel permeability which will result in the blood vessel leakage. It is this leak of blood vessel that will cause the resuscitated crystalloid fluid to pass from the blood vessel to the interstitial rapidly. This is thought to be the cause of the increase in the number of platelets under the normovolemia conditions.

CONCLUSION

The piglets used in this study have experienced an anemia condition since the beginning because the total value of the red blood cells of the pig has been below the normal range since before the treatment. The conditions of the hypovolemic shock and the hypervolemia resuscitation cause the significant decrease in all red blood cell variables except for the platelet values.

ACKNOWLEDGMENT

The authors acknowledge the support of the Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia for providing place for this research.

REFERENCES

- Bassols, A., C. Costa, P.D. Eckersall, J. Osada, J. Sabrià, and J. Tibau. 2014. The pig as an animal model for human pathologies: A proteomics perspective. *Proteomics Clin. Appl.* 8(9-10):715-731.
- Berndt, M.C. 2000. **Platelets, Thrombosis, and the Vessel Wall**. Harwood Academic Publishers, Amsterdam.
- Greer, J.P., J. Foerster, G.M. Rodgers, F. Paraskevas, B. Glader, D.A. Arber, and R.T. Means. 2009. **Wintrobe's Clinical Hematology**. 12th ed. Lippincott Williams and Wilkins, Philadelphia.
- Gutierrez, G., H.D. Reines, and M.E. Wulf-Gutierrez. 2004. Clinical review: Hemorrhagic shock. *Crit. Care*. 8(5):373-381.
- Kobayashi, L., T.W. Costantini, and R. Coimbra. 2012. Hypovolemic shock resuscitation. *Surg. Clin. N. Am.* 92:1403-1423.
- Kudo, D., Y. Yoshida, and S. Kushimoto. 2017. Permissive hypotension/hypotensive resuscitation and restricted/controlled resuscitation in patients with severe trauma. *J. Intensive Care*. 5(11): <https://doi.org/10.1186/s40560-016-0202-z>.
- Lunney, J.K. 2007. Advances in swine biomedical model genomics. *Int. J. Biol. Sci.* 3(3):179-184.
- Pascual, J.L., L.E. Ferri, A.J.E. Seely, G. Campisi, P. Chaudury, B. Giannias, D.C. Evans, T. Razek, R.P. Michel, and N.V. Christou. 2002. Hypertonic saline resuscitation of hemorrhagic shock diminishes neutrophil rolling and adherence to endothelium and reduces in vivo vascular leakage. *Ann. Surg.* 236(5):634-642.
- Perri, A.M., R.M. Friendship, J.C.S. Harding, and T.L. O'Sullivan. 2016. An investigation of iron deficiency and anemia in piglets and the effect of iron status at weaning on post-weaning performance. *J. Swine. Health Prod.* 24(1):10-20.
- Plowman, S.A. and D.L. Smith. 2008. **Exercise Physiology for Health, Fitness, and Performance**. 2nd ed. Lippincott William and Wilkins, Philadelphia.
- Rhoades, R.A. and D.R. Bell. 2009. **Medical Physiology: Principles for Clinical Medicine**. 3rd ed. Lippincott Williams & Wilkins, Baltimore.
- Rumbaut, R.E. and P. Thiagarajan. 2010. **Platelet-Vessel Wall Interactions in Hemostasis and Thrombosis**. Morgan and Claypool, California.
- Semiadi, G. and R.T.P. Nugraha. 2009. Some notes on biological aspects of captive javan warty pig (*Sus verrucosus*). *Biodiversitas*. 10(3):124-128.
- Spinella, P.C. and J.B. Holcomb. 2009. Resuscitation and transfusion principles for traumatic hemorrhagic shock. *Blood Reviews*. 23:231-240.
- Standl, T., T. Annecke, I. Cascorbi, A.R. Heller, A. Sabashnikov, and W. Teske. 2018. The nomenclature, definition and distinction of types of shock. *Dtsch. Arztebl. Int.* 115(45):757-768.
- Vincent, J.L. and D.D. Backer. 2013. Circulatory shock. *N. Engl. J. Med.* 369:1726-1734.
- Yoshida, T., M. Prudent, and A. D'alessandro. 2019. Red blood cell storage lesion: causes and potential clinical consequences. *Blood Transfus.* 17(1):27-52.