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Clinical and Hematological Parameters of Captive Timor Deer (*Rusa timorensis*) under Physical Restraint

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ABSTRACT

Timor deer (*Rusa timorensis*) is native species in Indonesia that faces many threatening issues. Studies related to the health aspects of Timor deer are considered essential to provide some information related to deer health management in captivity. Deer handling procedures in captivity in Indonesia are commonly carried out with physical restraint. Physical restraint in animals is known to cause various changes in animals' clinical and hematological values. This study aimed to determine the clinical and hematological parameters of Timor deer under physical restraint in captivity. This study used ten Timor deer that consisted of five stags and five hinds. The deer was positioned in a chute, then continued with the physical examination and blood sampling. Parameters observed in this study are clinical and hematological parameters. This study showed that clinical parameters of Timor deer under physical restraint have significant differences between sex groups, except for respiration rate parameters. Hematological parameters of physically restrained Timor deer did not show significant differences between sex groups, except for basophil parameters. This study concluded that physical restraint could affect the clinical and hematological parameters of Timor deer.

1. Introduction

Timor deer (*Rusa timorensis*) is native deer species of Indonesia. Timor deer are distributed naturally in Java and Bali and introduced to other parts of Indonesia (Fawzy *et al.* 2019; IUCN 2015). Timor deer is one of the nationally protected species based on its conservation status as vulnerable (IUCN 2015). One of the conservation efforts to save Timor deer from the threat of extinction is by developing *ex-situ* populations through a captive breeding program. Conservation agencies and individuals with special permission have developed many Timor deer breeding programs in Indonesia.

Studies related to deer health management are considered necessary in Timor deer conservation program. Aspects of deer health management need to have physiological reference values to evaluate the health condition of animals. Evaluation of animal health status can be done through physical examination and confirmed through laboratory examination. Through physical examination, clinical parameters, including heart rate, respiration rate, and body temperature, can be measured as an initial assessment of animal health conditions (Lovatt 2010). The hematological test is one of the standard tests to confirm the animal's health status through laboratory examination. Interpretation of the physical and laboratory examination results needs to be compared with the reported reference values. Until now, studies related to the physiological values of Timor deer have not been widely reported.

Studies related to the physiological values of Timor deer that have been reported only show the general physiological values without comparing between sexes. Animal handling factors also have a significant role in measuring physiological values in deer. Several studies found that deer under chemical immobilization showed changes in physiological and hematological parameters (Munerato *et al.* 2010; Topal *et al.* 2010). Generally, the chemical restraint with anesthetics agents is widely chosen to minimize the acute stress condition in deer (Caulkeet and Arnemo 2014).

Research and conservation activities involving wildlife capture require effective animal handling

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procedures and minimal risks to animals and operators. In Indonesia, many deer captivity has only used chemical restraint when the deer could not be handled by physical restraint or the deer needed to be immobilized for a particular purpose such as surgery. Chemical restraint requires anesthetic agents with the correct dose and must be supervised and handled by a veterinarian. Chemical restraint procedures also require operators who are experienced in using tools such as blowpipes or dart guns (Semiadi and Nugraha 2004).

The author's observation found that many deer captivity in Indonesia commonly used physical restraint rather than chemical restraint. This phenomenon is assumed to be due to more resources for physical restraint, such as rope or chutes. Considering physical restraint is the most common handling procedure in captive Timor deer, it is necessary to know the clinical and hematological values of Timor deer under physical restraint. This value is essential as a comparison in animal health assessment. This research aimed to study the clinical and hematological parameters of physically restrained Timor deer in captivity.

2. Materials and Methods

2.1. Location, Animals, and Ethical Research Approval

This study was done at the Bogor Development Center of Deer Captive Breeding Technology in Dramaga Research Forest, Indonesia. The captivity uses a semi-intensive system with each paddock connected to chutes by a gangway. This study used ten Timor deers aged between two and five years with weights ranging between 35 and 50 kg. Deer were grouped into two groups based on sex: five stags (velvet stage) and five hinds. The deer were proven clinically healthy by a veterinarian based on behavior observation, body condition score, fecal scoring, and negative blood parasite examination results. The deer used in this study were under permission from the Forest Research and Development Center Ministry of Environment and Forestry Republic of Indonesia. Procedures in this study were approved by Animal Care and Use Committee Faculty of Veterinary Medicine IPB University No. 021/KEH/SKE/XII/2020.

2.2. Handling Procedure

Handling procedures were done by positioning the deer in the chute. The deer was first positioned in the chute as physical restraint for the following examination. The chute used a drop-floor chute commonly used as a deer restraint cage. Deers reared in the paddock are herded into a gangway connected with a chute (Figure 1). Physical restraint was done under guidance from a qualified veterinarian and helped by experienced keepers to prevent unnecessary handling and minimize the potential injuries in animals.

2.3. Physical Examination

Physical examination procedures were carried out based on the method described by Casella

Figure 1. Physical restraint of Timor deer using the drop-floor chute

et al. (2016) and Kabuga (1992). The physical examination was carried out to determine the clinical parameters of animals, including the parameter of body temperature, heart rate, and respiration rate. Body temperature was carried out by per-rectal temperature measurement using a digital thermometer. Heart rate measurement was done by counting heartbeat through a stethoscope placed in the axilla region for one minute. The respiration rate was measured by counting the movement of thoracic and abdominal muscles for one minute.

2.4. Blood Sampling and Hematological Analysis

Blood was collected by jugular venipuncture using a disposable syringe and then stored in a K_3EDTA tube for hematological analysis. All collected blood samples were transported in an icebox and then analyzed at the Laboratory of Clinical Pathology, Faculty of Veterinary Medicine, IPB University. Collected blood samples were used to determine hematological parameters. Hematological parameters, including erythrocytes, leukocytes, and platelets, were determined using an automated hematology analyzer (VetScan HM5 Abaxis Inc).

2.5. Data Analysis

The clinical and hematological data results were analyzed based on Nigam *et al.* (2020). Data normality was analyzed using the Shapiro-Wilk test, and data homogeneity was analyzed using the Levene test. Data with normal distribution and homogenous were analyzed using an independent t-test to determine the differences between sex groups. Data without normal distribution but homogenous were analyzed using the Mann-Whitney test to determine the differences between sex groups.

3. Results

The clinical parameters of physically restrained Timor deer are presented in Table 1. The physical examination results showed that clinical parameters of Timor hinds were higher than Timor stags. Statistical analysis showed significant differences ($p<0.05$) between sex groups in body temperature and heart rate parameters. However, the respiration rate parameter result did not show significant differences between sex groups.

The results of the hematological analysis of physically restrained Timor deer are presented in Table 2. The statistical analysis results showed significant differences between Timor stags and hinds found in the basophil parameter. Hematological analysis results show that total erythrocytes, hemoglobin concentration, and hematocrit of Timor hinds are relatively higher than Timor stags. However, there are no significant differences between sex. Erythrocytes indices are relatively higher in Timor stags than Timor hinds, although there are no significant differences. The results of platelet parameters were relatively higher in Timor hinds except for platelet distribution widths which are relatively higher in Timor stags. The results of total and differential leukocytes are relatively higher in Timor hinds except for neutrophils which are relatively higher in Timor stags.

Table 1. Clinical parameters of captive Timor deer under physical restraint

Parameters	Unit	Sex				Combined	
		Stags		Hinds			
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Body temperature	°C	38.4±0.32[a]	38.0-38.8[a]	39.5±0.86[b]	38.1-40.4[b]	38.9±0.85	38.0–40.4
Heart rate	beat/minute	92.8±9.12[a]	84.0-108.0[a]	128.0±8.00[b]	116.0-136.0[b]	110.4±20.24	84.0–136.0
Respiration rate	breath/minute	39.2±7.16	32.0-48.0	48.0±4.90	44.0-56.0	43.6±7.41	32.0–56.0

[a,b]Numbers in the same row with different superscript indicates significant differences ($P<0.05$) between sex groups

Table 2 Hematological parameters of captive Timor deer under physical restraint

Parameters	Unit	Sex				Combined	
		Stags		Hinds			
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
RBC	10^6/µl	8.91±1.52	7.08-10.60	10.02±1.31	8.09-11.20	9.47±1.46	7.08-11.20
Hemoglobin	g/dl	12.44±1.08	10.60-13.30	13.12±0.66	12.00-13.70	12.78±0.92	10.60-13.70
Hematocrit	%	34.44±4.89	25.82-37.88	36.19±1.93	33.82-38.95	35.32±3.62	25.82-38.95
MCV	fl	39.00±6.24	34.00-48.00	36.60±3.44	34.00-42.00	37.80±4.92	34.00-48.00
MCH	pg	14.12±1.50	12.50-15.60	13.22±1.21	12.00-14.20	13.67±1.37	12.00-15.60
MCHC	g/dl	36.38±3.10	32.70-41.20	36.34±1.24	34.60-37.50	36.36±2.23	32.70-41.20
RDWc	%	23.78±1.36	22.40-25.20	23.82±0.95	22.30-24.60	23.80±1.11	22.30-25.20
Platelet	10^3/µl	128.00±47.95	95.00-183.00	231.80±108.19	94.00-380.00	192.88±101.15	94.00-380.00
PCT	%	0.08±0.03	0.06-0.11	0.17±0.15	0.05-0.42	0.14±0.12	0.05-0.42
MPV	fl	6.23±0.49	5.90-6.80	6.68±2.42	5.30-11.00	6.51±1.86	5.30-11.00
PDWc	%	31.27±1.01	30.20-32.20	28.86±5.55	19.00-31.70	29.76±4.41	19.00-32.20
WBC	10^3/µl	5.00±1.16	3.61-6.32	5.50±1.74	4.01-8.07	5.25±1.42	3.61-8.07
Lymphocyte	10^3/µl	1.25±0.62	0.63-2.16	1.60±0.71	0.82-2.45	1.43±0.65	0.63-2.45
Lymphocyte	%	25.56±12.52	14.26-40.00	32.42±19.75	14.87-55.86	28.99±16.00	14.26-55.86
Monocyte	10^3/µl	0.12±0.09	0.04-0.26	0.37±0.43	0.03-1.06	0.25±0.32	0.03-1.06
Monocyte	%	2.90±2.53	0.71-6.58	6.34±6.02	0.64-13.14	4.62±4.72	0.64-13.14
Neutrophil	10^3/µl	3.62±1.24	2.12-5.14	3.53±1.92	1.28-5.82	3.57±1.52	1.28-5.82
Neutrophil	%	71.55±13.41	53.67-83.73	61.26±20.67	31.92-79.35	66.41±17.30	31.92-83.73
Eosinophil	10^3/µl	0.10±0.08	0.05-0.24	0.17±0.18	0.06-0.49	0.14±0.14	0.05-0.49
Eosinophil	%	2.16±1.46	0.79-4.39	2.76±1.96	1.23-6.07	2.46±1.66	0.79-6.07
Basophil	10^3/µl	0.004±0.005[a]	0.00-0.01[a]	0.038±0.046[b]	0.01-0.12[b]	0.021±0.036	0.00-0.12
Basophil	%	0.09±0.12[a]	0.00-0.25[a]	0.60±0.51[b]	0.21-1.49[b]	0.34±0.44	0.00-1.49
N/L Ratio	-	3.57±2.00	1.34-5.87	2.84±1.96	0.57-4.85	3.21±1.91	0.57-5.87

[a,b]Numbers in the same row with different superscript indicates significant differences (P<0.05) between sex groups. RBC: red blood cell count, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDWc: red cell distribution width, PCT: platelet crit, MPV: mean platelet volume, PDWc: platelet distribution width; WBC: white blood cell count, N/L Ratio: neutrophil/lymphocyte ratio

4. Discussion

Many factors are known to play an essential role in the physiological parameters of Timor deer. Higher clinical and hematological values of Timor hinds than Timor stags are thought to be caused by various factors such as behavior, hormonal, and daily activities between the two sexes. Generally, female animals have higher physiological values in a higher body temperature, more frequent pulses, and a higher respiration frequency than male animals (Fadare *et al.* 2012).

Hematological values of deer have relation to body sizes and metabolic rates between sex. Animals with smaller body sizes have proportionally higher metabolic rates than larger animals. Higher metabolic rate consequences higher oxygen demand and increases total erythrocytes and hemoglobin concentration (Hawkey and Hart 1985; Rawson *et al.* 1992; Spaargaren 1994). Several studies showed that Timor hinds have significantly smaller body sizes than Timor stags (Maha *et al.* 2021; Woodford and Dunning 1992). These findings explain why Timor hinds have higher clinical parameters and erythrocyte values than Timor stags.

Behavior and daily activities also influence the physiological values of deer. Several studies showed that adult Timor hinds have relatively higher daily activities such as locomotion and feeding activities, followed by a lower percentage of resting activity than adult Timor stags (Fawzy *et al.* 2019; Pairah *et al.* 2014). The behavior and daily activities of Timor hinds are more active, resulting in higher physiological values of Timor hinds than Timor stags. The differences in physiological values between sexes are because the clinical parameters are closely related to animals' behavior (Marai *et al.* 2007; Tamioso *et al.* 2017). The higher daily activities in Timor hinds can affect the hematological values, such as higher total erythrocytes, higher hemoglobin concentration, and higher hematocrit values. The increases in heart and respiration rates affect erythrocyte parameters to conform to the oxygen demands.

The handling procedures play a significant role in the clinical values of Timor deer in this study. The clinical values of Timor deer under physical restraint in this study are generally different from the clinical values of Timor deer anesthetized intramuscularly with ketamine-xylazine as chemical restraint, as reported by Fitri *et al.* (2021). Timor deer with

physical restraint in this study had a heart rate ranging between 84-136 beats/minute and a respiration rate ranging between 32-56 breaths/minute, while a study from Fitri *et al.* (2021) on anesthetized Timor deer in Malaysia had a heart rate ranging between 40-60 beats/minute and a respiration rate of 30-40 breaths/minute.

Deers are easily excitable species, and physical restraint can lead to clinical and hematological changes in the deer (Boes 2010; Caulkeet and Arnemo 2014; Mariti *et al.* 2019). The use of deer chutes as physical restraint in this study can lead the acute stress condition that affects the increase of the clinical parameters of deer. Acute stress in animals can be associated with higher cortisol levels in the blood. Animals under stress will stimulate the hypothalamus to release corticotropin-releasing factor (CRF). CRF will stimulate the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland and eventually stimulate the release of cortisol in the blood (Afsal *et al.* 2018; Khodaei-Motlagh *et al*. 2011).

Higher cortisol levels in the blood caused by ACTH's hypersecretion will stimulate the adrenal gland hyperactivity, which stimulates the increase of epinephrine and norepinephrine in the blood. The increase in epinephrine and norepinephrine will stimulate animals' heart rate and respiration rate (Gruber *et al.* 2010). Increases in epinephrine levels can trigger increases in spleen contraction. Increased spleen contraction can lead to erythrocyte release in circulation and increase the hematocrit value (Jones and Allison 2007; Polizopoulou 2010). A study by Topal *et al*. (2010) on Red deer (*Cervus elaphus*) found that deer under physical restraint showed significantly higher clinical and hematological parameters than under chemical restraint, including heart rate, respiration rate, and hematocrit values.

In this study, total platelet counts of Timor deer were relatively low compared to total platelet counts of captive Timor deer in Thailand, which ranged between 132000–303000 platelets/µl (Salakij *et al.* 1999). The platelet counts of Timor deer in each sex group have a minimum value below 100000 platelets/µl, which can be considered thrombocytopenia (Jones and Allison 2007). However, our observation of Timor deer in this study did not find any clinical signs related to thrombocytopenia. In addition, lower platelet counts in deer were not associated with stress conditions caused by physical restraint. However, they were thought to be associated with the use of EDTA as an anticoagulant. EDTA as an anticoagulant can result in platelet clumping, which is erroneously counted by an automated hematology analyzer as a single platelet unit. This condition has been reported in several animals, such as horses, pigs, cats, and dogs (Russell 2010). Blood storage in EDTA for more than 4 hours can result in platelet clumping and swelling, resulting in lower total platelet counts and increased MPV values (Jones and Allison 2007; Roland *et al.* 2014).

Leukocyte profiles are generally related to ages, stress conditions, infection, and diseases and have no specific relation to sex differences (Boes 2010; Meneghini *et al.* 2016; Peinado *et al.* 1999). This statement aligns with a study from Ho *et al.* (2018) on captive Timor deer in Malaysia, which did not find significant differences in leukocyte parameters between sex groups. In this study, the effect of physical restraint is the most conncern in evaluating the leukocyte parameters of Timor Deer. These handling procedures can lead to acute stress in animals and change leukocyte parameters' results, especially in neutrophils and lymphocyte count. Other leukocyte parameters such as monocytes, eosinophils, and basophils are more related to diseases due to infection or immunity and less likely associated with stress conditions.

The neutrophil percentage of Timor deer in this study is higher than the neutrophil percentage of captive Timor deer in New Caledonia, which ranged from 34% to 54%. The lymphocyte percentage of the Timor deer in this study is slightly lower than the percentage of lymphocytes of the captive Timor deer in New Caledonia, which ranged from 34 to 52% (Audigé 1992). Higher neutrophil count and lower lymphocyte count in this study were caused by differences in the handling procedures when taking blood samples. In this study, handling procedures of Timor deer used physical restraint while handling in Audigé's study (1992) used xylazine as a chemical restraint. Physical restraint used in this study can lead to acute stress conditions in deer and increase neutrophils, followed by a decrease in lymphocytes in circulation.

Physical restraint in deer can lead to acute stress conditions, significantly increasing the total leukocytes in circulation due to catecholamine and glucocorticoid releases (Munerato *et al.* 2010; Marco and Lavín 1999). Increased catecholamine and glucocorticoids result in lymphocyte destruction in the thymus cortex, stimulating the mobilization of neutrophils from the marginal pool to the circulation pool, prolonging neutrophil life in circulation, and affecting the higher ratio of neutrophils to lymphocytes (Kim *et al.* 2005; Marco and Lavín 1999).

Stress conditions in animals can be evaluated from the ratio of neutrophils to lymphocytes (N/L ratio) (Davis *et al.* 2018). Several studies found that

neutrophil or heterophil to lymphocyte ratios are more practical for measuring stress in animals than other measurements (Cīrule *et al.* 2012; Johnstone *et al.* 2012). Neutrophil to lymphocyte ratios in deer under normal conditions are typically equal (Boes 2010). A study from Kannan *et al.* (2000) found that N/L ratios higher than 1.5 indicate that animals were under stress. The average N/L ratio of Timor deer in this study is 3.21±1.91, proving that the deer were under stress conditions caused by physical restraint. However, there were no significant differences in the N/L ratio between sex groups.

Physical restraint generally can cause acute stress and lead to other pathological conditions such as capture myopathy in deer. Prevention of capture myopathy in deer can be done by minimizing duration and restraint times. The use of sedative agents can be chosen to reduce the potential for capture myopathy during physical restraint (Breed *et al.* 2019; Caulkeet and Arnemo 2014; Cattet *et al.* 2004). We recommended that physical restraint be done effectively to minimize unnecessary action that can cause deer more stress. The use of physical restraint is also required to notify the duration and frequency of handling of deer. We also recommend using chemical restraint rather than physical restraint to minimize the changes in clinical and hematological parameters due to stress that can lead to pathological conditions in deer.

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