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COMPARISON ON STRESS LEVEL BETWEEN SEDATED AND NON SEDATED DOG FOR EUTHANIZING PROCESS IN DBKL

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COMPARISON ON STRESS LEVEL BETWEEN SEDATED AND NON SEDATED DOG FOR EUTHANIZING PROCESS IN DBKL

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CERTIFICATION

It is hereby certified that we have read this project paper entitled " Comparison on Stress Level Between Sedated and Non-sedated Dogs for Euthanizing Process in DBKL," by Mohammad Ikram Bin Ahmad Fuzi and in our opinion it is satisfactory in terms of scope, quality and presentation ad partial fulfilment of the requirement for the course VPD 4999 – Project.

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DEDICATION

I dedicate this thesis to:

My dearest family

HJH. NASHITAH BINTI HAJI ABDULLAH

NORALIYA BINTI AHMAD FUZI

MOHAMAD IHSAN BIN AHMAD FUZI

NASRUL HAKIM BIN AHMAD FUZI

The one that I love

SYAHIRATUN NABIHAH BINTI CHE OMAR

My supervisor

PROF DR MD ZUKI ABU BAKAR

My co-supervisor

DR. HAZILAWATI HAMZAH

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada kepreluan kursus VPD 4999 – Projek

PERBANDINGAN TAHAP TEKANAN KEPADA ANJING YANG DIBERI UBAT PELALI DAN TANPA UBAT PELALI DALAM PROSES EUTHANASIA DI DBKL

By,

Mohammad Ikram Bin Ahmad Fuzi

2017

Penyelia: Prof. Dr. Md. Zuki Abu Bakar
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Dr. Hazilawati Hamzah

Prosedur euthanasia adalah prosedur biasa yang telah dijalankan di DBKL untuk memusnahkan anjing- anjing liar. Anjing tersebut mungkin akan mengalami tekanan yang besar semasa menjalani proses euthanasia. Objektif kajian ini adalah untuk menilai kepekatan serum kortisol dan nisbah neutrophil kepada lymphocyte untuk prosedur euthanasia pada anjing yang diberi ubat pelali dan tanpa ubat pelali sebagai mengukur tahap tekanan. Sebanyak 40 anjing dewasa, 20 betina dan 20 jantan dari DBKL, berat badan 8-15 kg anjing telah digunakan dalam kajian ini. Darah dikumpulkan secara intravena melalui urat lehernya. 10 ajing jantan dan 10 anjing betina telah diberi ubat pelali, manakala 10 anjing jantan dan

anjing betina yang lain tidak diberi ubat pelali. Serum kepekatan kortisol dan nisbah neutrophil kepada lymphocyte telah ditentukan dengan menggunakan radioimmunocerakinan dan pengkamiran kiraan. Hasil kajian menunjukkan terdapat perbezaan signifikan ($P < 0.05$) dalam nisbah neutrophil kepada lymphocyte antara anjing dengan ubat pelali dan tanpa ubat pelali. Walau bagaimanapun, bagi kepekatan serum kortisol, tidak ada perbezaan yang signifikan ($P > 0.05$) antara anjing dengan ubat pelali dan tanpa ubat pelali. Walau bagaimanapun, bermakna pangkat untuk anjing tanpa ubat pelali adalah lebih tinggi daripada anjing dengan ubat pelali di kedua-dua kortisol dan nisbah neutrophil kepada lymphocyte. Ini menyimpulkan bahawa ubat pelali menggunakan acperomazine boleh mengurangkan tindak balas tekanan kepada anjing untuk menjalani proses euthanasia

Kata kunci: euthanasia, ubat pelali, tekanan, kortisol, nisbah neutrophil kepada lymphocyte

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 project.

COMPARISON ON STRESS LEVEL BETWEEN SEDATED AND NON SEDATED DOGS FOR EUTHANIZING PROCESS IN DBKL

by

Mohammad Ikram bin Ahmad Fuzi

2017

Supervisor: Prof. Dr. Md. Zuki Abu Bakar
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Dr. Hazilawati Hamzah

Euthanizing procedure is a common procedure that was conducted in DBKL to cull the stray dogs. The dog may having enormous stress during euthanizing procedure. The objective of this study was to evaluate the serum cortisol concentration and neutrophil to lymphocyte ration following euthanizing procedure in sedated and non-sedated dogs as an indicator of stress level. A total of 40 adult dogs, 20 females and 20 males from DBKL, weight 8-15 kg were used in this study. Blood was collected intravenously through jugular vein. Ten male and 10 female dogs were sedated using acepromazine prior to euthanasia, while another 10 male and 10 female dogs were not sedated. Serum cortisol concentration and neutrophil to lymphocyte count were determined using radioimmunoassay and differential count, respectively. The results revealed that there was significant difference ($P<0.05$) in the neutrophil to lymphocyte ratio between sedated and non-sedated dogs. However, for the serum cortisol concentration, there was no significant difference ($P>0.05$) between the sedated and non-sedated dogs.

However, mean rank for non sedated is higher than sedated animal on both cortisol and also neutrophil lymphocyte ratio. This conclude that sedation using acepromazine can reduced stress response in dogs prior enthanizing process.

Keywords: euthanize, sedation, stress, cortisol, neutrophil to lymphocyte ratio

CHAPTER 1

INTRODUCTION

Euthanasia word originated from the Greek word which is ‘eu’ means ‘good’ and ‘thanatos’ means death and when literally translated it means ‘good death’. There are four primary criteria that ensure death cause by methods of euthanasia is humane (Beaver et al., 2001). The four criteria is the method must be painless, achieve rapid unconsciousness followed by death, minimise animal fear and distress and be reliable and irreversible.

The dog may have stress due to restraining and euthanizing process. Thus, the idea is to use Acepromazine as sedative agent to the dog before euthanizing was done to reduce stress. Acepromazine is one of the most-commonly used tranquilizers for dogs and cats. It decreases anxiety, causes central nervous system depression and a drop in blood pressure and heart rate (Barbara, 2013). The dog will more calm and relax and easier to restrain. If the dog is too fractious the sedation will help so that the animal can be handled safely.

In 1936, Selye reported that stress is used to describe a nonspecific response of the organism to noxious stimuli. Various definitions of stress are made subsequently (Selye, 1952; Perry, 1975; Broom, 1988 and Moberg et al., 2001) that most of the author describes stress as response of organism to an internal or external threat where it concentrates effort to cope with. Normally, stress is caused by negative factors (Nagel and Reinhardt, 2003). The word stress is widely used, but no clear definitions has been accepted. However, it is often defined as when an animal is suffering a threat to its homeostasis, and the threat is defined as “stressor”. According to Moberg (2000), if an animal is failing to cope with stress, that is if the stress

response gives no reduction of the amount of stress, therefore, the animal will experience distress and decrease its welfare.

In recent decades, measuring cortisol level has become increasingly popular in field research and clinical study aimed to investigate bodily response to psychosocial stress and other psychological and clinical condition. Cortisol in major glucocorticoid product of adrenal cortex (Turpeinen et al, 2013). The role of cortisol is divided into 2 main areas which is metabolism and inflammation. In other words, cortisol can be used to measure pain and distress (Kristenson, 2012). The release of cortisol is mediated by the Hypothalamus-Pituitary-Adrenal (HPA) axis. The principal stimulus of the HPA axis is Corticotrophin-Releasing Hormone (CRH), which is locally produced in hypothalamus (Kristenson, 2012).

Besides that, to measure stress, we can also used haematological parameters such as white blood cell count through blood smear (Davis et al., 2008). Measuring neutrophil lymphocyte ratio is simple, inexpensive and suitable for routine use to obtain information about stress and psychopathological symptoms (Press, 2016). Maheshwari et al. (2013) also determined that we can use neutrophil lymphocyte ratio as stress indicator. Combination of both parameter (cortisol level and neutrophil to lymphocyte ratio) has been uses to assess stress in transportation (Maheshwari, 2013).

Euthanizing procedure may cause stress and pain to the dogs and this might be concern to welfare of the animal. No study has been conducted to measure stress response in dogs during euthanizing process in DBKL. Therefore, this study was conducted on sedated and non-sedated dogs and compared the stress response between the two groups.

HYPOTHESIS

Sedated dogs have lower stress response compare with non-sedated dogs during euthanizing process.

OBJECTIVES

The objective of this study is to evaluate stress response during euthanizing process by comparing plasma cortisol concentration and also neutrophil-lymphocyte ratio in sedated and non-sedated dogs.

CHAPTER 2

LITERATURE REVIEW

2.1 Stress and Animal Welfare

There are variety of measurement that is important in accessing welfare which includes health (Alban and Agger, 1997), immunology (Amadori et al., 1997), physiology (Jacobson and Cook, 1998) and behaviour (Wiepkema 1983). Welfare can be referred to the physiological state of the animal that related to its external and internal environment (Fraser 1999). The environmental stimuli that affect the homeostasis is known as stressor and the reaction of animals as defence is known as stress response (Mostle and Palme 2002).

Poor welfare of animal is considered a condition when the animal becomes more susceptible to negative pathologies (Moberg, 1993). Poor animal welfare is when the animal is unable to response according to their biological nature in their species (Rollin, 1995).

If an animal is failing to cope with stress, that is if stress response gives no reduction of the amount of stress, then the animal experience distress and it will decrease its welfare (Moberg, 2000). When the biological system fails to cope with the stressor(s) and behavioural activity is suppressed, an animal depends on the integrative capacities of nervous and endocrine systems (Zulkifli et al., 2013).

2.2 Stress Response

According to Matteri (2000), there are various hormones that involved is stress response such as ACTH, glucocorticoids, prolactin and catecholamines. Balm (1999), stated in term of physiology, stress response is possible with discovery of metabolic, endocrine and immunological mechanism. Stress can be caused by physical or environmental, psychological and physiological stressor. The physical stressors such as pain, disease, temperature and deprivation of feed and water. Examples of psychological stressors are fear, restraint, isolation and presence of humans (Dantzer et al., 1983).

Hypothalamic-pituitary adrenal (HPA) axis and the sympathetic nervous system are the two areas in the brain that are affected by a stress response. This will cause the release of glucocorticoids from the adrenal gland through the stimulation of the HPA and the release of norepinephrine through the stimulation of the sympathetic nervous systems (Tilbrook and Clark 2006). Dogs are affected by many of these psychological stressors, most notably novel, unpredictable or uncontrollable situations that influence HPA (Coppola et al., 2006). Increase in HPA activity can be used as parameter to measure stress which measure corticosteroid that was released in the blood when stimulated by stressors (Sapolsky et al., 2000).

HPA axis is responsible for neuroendocrine adaptation component of stress response. This response is characterized by hypothalamic release of corticotropin-releasing factor (CRF). CRF is also known as CRH or corticotropin-releasing hormone. When CRF binds to CRF receptors on the anterior pituitary gland, adrenocorticotropic hormone (ACTH) is released. ACTH binds to receptors on the adrenal cortex and stimulates adrenal release of cortisol (Alschuler, 2016). Moemede et al. (1980) stated that they can also measure activation of HPA axis which is elevation of plasma glucose level that are affected by released of glucocorticoid.

2.3 Stress and Immune Response

Two non-exclusive hypotheses have been proposed. One proposes that stress hormones tend to suppress immune function, so that an individual's resources are preferentially allocated to processes more crucial for surviving the stressor. The other proposes that attenuation of immune activities in response to stressors keeps the body from harming itself. During stress responses, many host-derived, novel molecules (e.g., antigens) are revealed to the immune system, and stress hormones prevent immune cells from mounting misdirected attacks (Råberg *et al*. 1998).

2.4 Cortisol

According to Turpeinen (2013), cortisol is natural glucocorticoid hormone that is routinely released by the adrenal cortex. Hypothalamic corticotrophin releasing hormone (CRH) and adrenocorticotropic (ACTH) will stimulate cortisol production from the hypophysis. Cortisol is recognized as major indicator to measure stress and is used in animal welfare to measure stress (Blackshaw et al., 1989). The level of cortisol will usually rise in the blood circulation in response to stressful situation caused by stimuli (Randall, 2012). Turpeinen (2013) also stated that cortisol main function is to restore homeostasis following exposure to stress. In addition, Mostl et al. (2002) said that increase in cortisol or glucocorticoid secretion is one of the mechanisms of defence against stressful condition regulated by endocrine system.

In dogs, there is no documented difference in basal levels of cortisol between sexes. Basal cortisol levels are influenced by size and age not reproductive status (Reimers et al., 1990). Stress induced cortisol levels do not follow the same trends. In stress situations, females tend to have higher cortisol level than male (Reimers et al., 1989). In addition, Hennessy (1989) said that juvenile/pups have lower cortisol compare with adults.

2.5 Neutrophil to Lymphocyte Ratio

Leucocyte profiles are very useful in field because they can be directly related to stress hormone levels and can be altered by stress. Dhabhar et al. (1996) stated that leucocyte profiles that deviate from normal parameter were routinely used to indicate mammalian hormonal stress responses in 1940s, before methods were available directly assess plasma glucocorticoids. Research was conducted using adrenocorticotrophic hormone (ACTH) in boars (Bilandzic et al., 2006) and horses (Rossdale, Burguez and Cash, 1982) to stimulate release of glucocorticoid from adrenal glands. It shows treatment with ACTH increases neutrophil to lymphocyte ratio probably via effects on glucocorticoid secretion.

Long terms of leucocyte in blood are seen in humans with chronic medical disorders. The patients that have chronic medical disorders will have elevated neutrophil counts and lower lymphocyte count when compared to healthy individuals (Albright et al., 1946). Chronically elevated glucocorticoids may cause long-term elevation in neutrophil to lymphocyte ratio.

According to Dhabbar (2002), stress induce reductions in circulating lymphocyte number are not due to large scale destruction of cells, but rather to glucocorticoid induced

alterations in ‘trafficking’, or redistribution of lymphocyte from the blood to other body compartments. Glucocorticoids will stimulate influx of neutrophil into the blood from bone marrow that different type of cells are routed where they are needed during stress response (Bishop et al., 1968)

CHAPTER 3

MATERIALS AND METHODS

3.1 Animals

This study was done in early January 2017. The animal used were the stray dogs from Unit Kawalan Vektor under Dewan Bandaraya Kuala Lumpur, Kuala Lumpur. The number of animals used was 20 male and 20 female adult dogs with approximately similar body weight (8 - 12 kg) and good body condition. The dogs used in this study were all culled dogs subjected for euthanasia. The dogs were restrained properly during the process by personnel from DBKL using the snare. The blood was collected while the dog was restrained and euthanizing was done right after the sampling was done.

Out of 20 male and 20 female dogs, 10 male and 10 females were given sedation. Sedative drugs used was Acepromazine, 0.1 mg/kg and was administered subcutaneously using 3 ml syringe and 21G needle. The concentration of the drug was 5 mg/ml. Acepromazine can be an effective tranquilizer and can control overly excitable animal. The blood was collected after 30 minutes sedation was done. The blood was collected with aid of restraining method using snare by DBKL personnel.

3.3 Blood Sampling

The blood sample were collected by venepuncture using 23 Gauge needle from jugular vein before euthanizing was done. The blood were collected both from two groups which is sedated and non-sedated dogs. The dogs were restrained using snare during blood collection. EDTA and plain tubes were used to collect the blood samples. The blood sample collected in EDTA tube was used for blood smear to determine neutrophil to lymphocyte ratio. The blood also used to check pack cell volume (PCV) and plasma protein of the blood using microcentrifuge (Haematokrit 20 – Hettich instruments). Scil Vet ABC ^{TM}Hematology Analyzer was used to determine total number of white blood cell (WBC), red blood cell (RBC), haemoglobin and also platelets in blood. The blood sample from plain tubes (serum) was used to measure serum cortisol concentration. The plain tubes were centrifuged (at 5000 revolution per minutes for 15 minutes) at room temperature to separate the serum in the blood. The serum then was aspirated using pipette and transferred into 1.5ml eppendorf tubes and kept frozen at -20 °C for further assay analysis.

3.4 Neutrophil to Lymphocyte Ratio

Thin blood smear was done on the glass slide according to standard method. The blood smear was done at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The thin blood smear was stained using Wright's stain for 3 minutes and buffer solution for 8 minutes. The back of slide then was rub using gauze and let it dry in room temperature. The slides was examined under light microscope (Nikon Eclipse E200) using x40 magnification and differential count was determined by counting up to 200 cells white blood cell. Then, the neutrophil to lymphocyte ratio was calculated.

3.5 Cortisol Concentration

The measurement of cortisol concentration was determined by using Cortisol Radioimmunoassay kit produced by Beckman Coulter. The frozen serum must be thawed at room temperature. Then, 50µL of serum and 500µL of tracer was added into each antibody-coated tubed and mixed gently using vortex mixer (IKA-Vortex 1). The tubes then were incubated at room temperature on the orbital shaker (IKA-Vibrax-VXR) for 1 hour. Then, the tubes were placed into sponge rack and placed into inverted position to drain all the contents of the tubes. Lastly all the tubes were placed into 1470 automatic gamma counter (WIZARD®) for cortisol concentration measurement.

3.6 Statistical Analysis

With the data collection, the data was further analyse using SPSS Version 22. Normality test was done to determine a suitable test that can be used for the data. The data was not normally distributed and the test chosen was Mann-Whitney test. Mann-Whitney test was used to compare between sedated and non-sedated dogs in comparison of cortisol concentration and also neutrophil-lymphocyte ratio.

CHAPTER 4

RESULTS

4.1 Comparison on Serum Cortisol Concentration and Neutrophil-Lymphocyte Ratio in Sedated and Non-sedated Dogs

Table 1 shows statistical analysis for serum cortisol concentration and neutrophil: lymphocyte ratio. Table 2 shows the value of mean rank in neutrophil:lymphocyte ratio and serum cortisol concentration in sedated and non-sedated dogs. For neutrophil:lymphocyte ratio, there was a significant different ($P<0.05$), but not for cortisol concentration. The mean rank for non-sedated dogs is significantly higher (24.13) compared to the mean rank for sedated dogs (16.88). It showed that non-sedated dogs were significantly stress compared with the sedated dogs. However, the cortisol concentration was not significantly different ($P>0.05$) when compare between the two groups. However, as it showed by the mean rank, the non-sedated dogs have higher mean rank (21.55) compared to non-sedated dogs (19.45). This indicates that non-sedated dogs has higher cortisol concentration compared with sedated dogs.

Test Statistics[a]		
	Neutrofil:lymphocyte ration	Cortisol
Mann-Whitney U	127.500	179.000
Wilcoxon W	337.500	389.000
Z	-1.961	-.568
Asymp. Sig. (2-tailed)	.050	.570
Exact Sig. [2*(1-tailed Sig.)]	.049[b]	.583[b]

Table 1: Test statistics for neutrophil:lymphocyte ratio and cortisol concentration.

Groups		N	Mean Rank	Sum of Ranks
Neutrofil:lymphocyte ration	Sedated dogs	20	16.88	337.50
	Non-sedated dogs	20	24.13	482.50
	Total	40		
Cortisol	Sedated dogs	20	19.45	389.00
	Non-sedated dogs	20	21.55	431.00
	Total	40		

Table 2: The mean rank of both neutrophil:lymphocyte ratio and serum cortisol concentration in non-sedated dogs.

4.2 Neutrophil Count in Sedated and Non-sedated Dogs

Neutrophil count was calculated manually using data gained from number of neutrophil count from neutrophil:lymphocyte ratio and also total number of white blood cell. This was done to determine if the dog having neutrophilia or have normal neutrophil count. Normal neutrophil count for canine species is between 3-11.5. When the value exceeds 11.5, it is considered neutrophilia. The results revealed that 9 out of 20 sedated dogs were neutrophilia and the remained 11 sedated dogs have normal neutrophil count. For non-sedated dogs, 3 out of 10 dogs were neutrophilia and remaining 7 have normal neutrophil count. Table 3 shows that cortisol level in sedated dogs either with high or normal neutrophil count were not significantly different ($P>0.05$). However, the cortisol level in sedated dogs with high neutrophil count were significantly elevated compared to dogs with normal neutrophil count indicated the dogs having stress ($P>0.05$). For neutrophil to lymphocyte ratio, both sedated and non-sedated dogs with high or normal neutrophil count were not significantly different ($P>0.05$).

Ranks					
Groups			**N**	**Mean Rank**	**P value**
Sedated dogs	Neutrofil:lymphocyte ratio	Neutrophilia	9	12.28	0.224
		Normal neutrophil count.	11	9.05	
		Total	20		
	Cortisol	Neutrophilia	9	12.78	0.119
		Normal neutrophil count.	11	8.64	
		Total	20		
Non-sedated dogs	Neutrofil:lymphocyte ratio	Neutrophilia	3	5.67	0.909
		Normal neutrophil count.	7	5.43	
		Total	10		
	Cortisol	Neutrophilia	3	9.00	0.017
		Normal neutrophil count.	7	4.00	
		Total	10		

Table 3: The neutrophil:lymphocyte ratio and cortisol concentration in sedated and non-sedated dogs.

CHAPTER 5

DISCUSSION

From the findings in this study, it revealed that sedated dogs having less stress during euthanasia process performed in DBKL compared with the non-sedative dogs. Although the cortisol concentration was not significantly different between the two groups, but the mean rank for non-sedated dogs was higher that the sedated dogs on both cortisol concentration and neutrophil to lymphocyte ratio suggesting that the non-sedated dogs was more stressful during euthanizing process compared with the sedated dogs. Thus, the use of sedative to reduce stress in dogs during euthanasia process is necessary. Lopez Olvera et al. (2007) use the Acepromazine to improve the welfare of captured Southern chamois by decreasing the risk of stress-related conditions. Another research earlier done by Lopez Olvera et al. (2005) reported that Acepromazine can reduce the adverse consequences of transport stress, as demonstrated by heart rate, body temperature, cortisol, creatinine, muscular enzyme, urea, sodium and potassium.

However, the effect of acepromazine to reduce stress was slightly contradictory. Bergeron et al. (2002) found that acepromazine does not changed the increase in cortisol level induced by air transport in dogs.

Whereas, in neutrophil to lymphocyte ratio, the results revealed a significant different ($P>0.05$) between the sedated and non-sedated groups. Acepromazine increases the splenic sequestration of red cells (Plumb, 2002) and, consequently, will markedly decrease RBC, PCV, haemoglobin concentration and lymphocytes while inducing a smaller increase in WBC and

neutrophils. Acepromazine minimise the increase of haematological values due to the stress of capture and handling (Lopez Olvera et al., 2007).

Some study stated that neutrophil to lymphocyte ratio is more reliable to measure chronic stress. According to Romero and Reed (2005), neutrophil to lymphocyte ratio tend to persist longer compared to changes in level of glucocorticoids hormone which can return to its baseline less than 1 hour. Neutrophil to lymphocyte ratio are able to persist for several hours (Dhabbar et al., 1996).

The dogs used in this study were stray dogs, therefore the dogs may have many underlying diseases that may disturb the accuracy of the results. Underlying disease is one of the stressor that will affect the result. Furthermore, the animal also may having stress when it was in captivity. In the shelter, socialization with other dogs and human is essential for good psychological well-being. Social isolation or restriction is regarded as a major stressor for social species like the dog (Wolfe, 1990).

Coppola et al. (2005) stated that when the dogs in the shelter, animal that engaged in a human contact session had lower cortisol level on day 3 than those did not. It means that the dogs need 3 days to suite itself in the shelter. Before 3 days, the dogs will having stress when it was keep in the shelter. Different time period the dogs stay in the shelter could cause different level of stress.

Furthermore, from the results of this study, it also revealed that the cortisol level in the non-sedated dogs with high neutrophil count significantly elevated compared to the dogs with normal neutrophil count. This means that underlying disease from the dogs may cause the

release of cortisol in blood due to stress. The results also showed similar condition in the sedated dogs. Although it was not significantly different, we can still take the value from the mean rank that showed the cortisol level in the dogs with neutrophilia was higher.

Previous research done in dairy cattle during dehorning, demonstrated an increase in the neutrophil to lymphocyte ratio which paralleled to an increase in cortisol (Doherty et al., 2007), similarly to pigs exposed to a transport stressor (McGlone et al., 1993). Furthermore, according to Morrow-tesch et al. (1993) and Kim et al. (2005), there are similar correlation between elevated cortisol concentrations and increase neutrophil to lymphocyte ratio in captive primate. This support that neutrophil lymphocyte ratio is reliable to measure stress response.

Although measurement of cortisol concentration in blood plasma would be a good indicator of stress, the neutrophil to lymphocyte ratio can also be used to measure acute stress as revealed by the results in this study. This parameter may be useful to study the effects of stress on the welfare of the animal. Acepromazine can be a good sedative to lower stress response in dogs prior euthanizing process, therefore increase the welfare of the animal following euthanizing process.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

In summary, cortisol level was not significantly different between sedated and non-sedated dogs. However, there was significant different in neutrophil lymphocyte ration between sedated and non-sedated dogs. The mean rank for non-sedated animal is higher than sedated animal on both cortisol and also neutrophil lymphocyte ratio. This indicate non-sedated animal is more stressful and having pain compare with sedated animal. The study also shows that the cortisol level in non-sedated dogs with high neutrophil count significantly elevated compared to dogs with normal neutrophil count also indicate more stress in non-sedated dogs. We can conclude that sedation using Acepromazine can educed the stress response in dogs prior euthanizing process.

For further study in the future, it is recommended to increase sample size to obtain more reliable and concrete results. Confirm health condition of the animal would be good. Therefore, the result will be more accurate in evaluating cortisol level and eliminate other factors that can cause increase in cortisol level such as present of underlying disease.

Furthermore, further research using other sedatives or tranquilizers should be done. Hence, we can determine the best sedatives agent and best dosage of drugs that we could use to sedate the dogs to minimise stress response in the dogs prior euthanizing process.

Neutrophil to lymphocyte ratio can be alternative method to measure stress response in period of time. A large body of evidence demonstrate that the adrenal and leucocyte response to stress are tightly linked. Neutrophil to lymphocyte would offer more to save money and time. Furthermore, it is a low cost procedure and only need to do white blood cell count on a blood smear using microscope. Measurement of cortisol would require expensive test kit such

as ELISA or radioimmunoassay kit. It would be more time consuming and limited compared if we use white blood cell count to determine neutrophil to lymphocyte ratio.

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APPENDICES

Appendix A : Kennel at DBKL

Appendix B : Sedative drug

Acepromazine